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JOHS. SCHMIDT:

INVESTIGATIONS ON HOPS

(*Humulus lupulus*, L.).

III. THE POLLINATION AND FERTILIZATION PROCESSES IN HUMULUS LUPULUS L. AND H. JAPONICUS SIEB. ET ZUCC.

With 22 text-figures and 2 double Plates.

BY

Ö. WINGE.

(Received for publication, June 1914.)

1. Introduction.

To understand the biology of the hops plant it was of importance to know the phenomena connected with the pollination, fertilization and development of the fruit and during the years 1911—14 at the instigation of Dr. Johs. Schmidt I have carried out a number of observations on the hops, both in the open and more especially by means of the microscope.

The question of the fertilization is for hops growers of great importance, though opinions are greatly divided with regard to the influence of the fertilization on the commercial and brewing value of the trade hops. In Germany and Austria it is made an essential requisite of good hops, that it contains no hops fruits; on the other hand, in England, Belgium, America and Australia no stress is laid on this. The consequence of this again is, that the male plants in the first two countries are destroyed with might and main, at some places even it is forbidden by law to have the male plants growing in the fields, — whilst hops growers in the last-mentioned countries deliberately keep the male plants in the cultures in order that the catkins, being stimulated in their growth by the fertilization, may attain to a greater luxuriance in appearance and a heavier weight. On this point at present it is a case of opinion against opinion. So much however is known with certainty, that the catkins become larger and heavier,

the more fertilized flowers there are, and that this increased weight is not due merely to the weight of the fruit.

It was also of importance to ascertain in this connection, how far the view of Wettstein (1903) was correct, that the hops may occasionally develop fruit parthenogenetically. Frequent reports have appeared from the hops districts of Germany and Austria, that in spite of all regulations to prevent the formation of the fruit in the hops, this never quite succeeds. Even at places where it was quite certain, that no male plants could be in the neighbourhood, it has proved that a small percentage of the catkins contained fruits. It is presumably these reports, which have induced Wettstein to put forward his, certainly cautious, statement regarding parthenogenesis.

There is also the possibility, that quite different plants may be able to fertilize the hops, a view put forward already in 1883 by a Bavarian hops grower (Allg. Br. u. Hopfen Zeitung II, p. 630), referring specially to the nettle *(Urtica)*, — and this question will likewise be mentioned in the following.

The following pages thus deal for the most part with a small section of the yearly ontogeny of the hops plant, as we only take account of the phenomena in question from the time, when the flowers are about to form the sexual cells until fertilization has occurred and the germ is developing. On the basis of cytological investigations and pollen experiments we discuss the phenomena which take place or may take place in both the male and female hops flowers, without taking count of the general morphology of the plant, branching of the flower stalks and the like, subjects which, especially as regards the female plant, have been closely investigated by Wydler (1844), Irmisch (1848), Lermer and Holzner (1892, I and II) and by Zinger (1898).

2. Earlier information regarding the pollination and fertilization processes in the hops.

Our knowledge of the pollination and fertilization of the hops is already considerable, chiefly through Zinger's work (l. c.); but some of this author's statements I find in disagreement with the actual facts. Zinger has endeavoured to show, how the pollen grain develops on the styles of the female plant and the manner in which the pollen tube penetrates down to

the female cells. It is just in these phenomena and in Zinger's statements regarding the structure of the integuments, that I find some divergence. Much regarding the growth of the pollen tube has been studied, though the investigation resulted, however, in a somewhat misleading description, but no one has hitherto taken up the more detailed study of the nuclei, if we except a small contribution from Modilewsky (1908). Zinger's main results with regard to the pollination and fertilization of the hops, including also *Humulus Japonicus* and *Cannabis sativa*, are brougth together by the author himself in the following passage (pag. 248):

"Die beiden Integumente verwachsen mit einander von der Hinterseite der Samenknospe, und die Mikropyle wird bei den Cannabineen obliterirt; diese Eigentümlichkeit muss als eine Anpassung, die zur Leitung des Pollenschlauches dient, betrachtet werden.

Der Pollenschlauch der Cannabineen, nachdem er in den Griffel gelangt ist, wachst fortwährend innerhalb des Gewebes des Fruchtknotens und erreicht den Nucellus der Samenknospen ohne in die Fruchtknoten[1]) zu gelangen.

Die Placentation wie auch das Verhalten des Pollenschlauches bei den Cannabineen deuten darauf hin, dass diese Pflanzen von den porogamen Pflanzen, deren Fruchtknoten viel complicirter gebaut ist, keineswegs abgeleitet werden können."

Zinger's above-mentioned work agrees closely with Nawaschin's investigations (1894) on chalazogamy in *Betula* etc., and in the passage of the pollen tube in the Cannabinae he finds great resemblance especially with *Ulmus*, in which the condition has been described by Nawaschin.

With regard to the oogenesis, we find in Lermer and Holzner (1892, II) only a statement of the presence of antipodal cells in the embryo-sac, whilst Zinger in his paper deals with the matter more in detail and accompanies his description with illustrations.

In Modilewsky's above-mentioned paper the nuclear condition in the embryo-sac is lightly touched upon and the author, who however deals mostly with the Urticaceae, states that he finds in *H. Japonicus*, that the embryo-sac contains two kinds of endosperms, a matter on which I disagree with the author.

---

[1]) Should naturally be "Fruchtknotenhöhle." *Ö. W.*

Modilewsky's results will be dealt with more closely at the end of chapter 7.

In a footnote Zinger l. c. states, that he made his investigations partly directly on young ovaries by using media for clearing the tissues, by which means he was able conveniently to follow the successive cell divisions, partly also by means of microtome series. The contents of the embryo-sac in the various stages of development were sufficiently well fixed in an alcoholic solution of acetic acid-sublimate. The objects were stained in toto in saffronin and the sections thereafter dipped in haematoxylin.

Our own methods will be seen from the following chapter.

3. Methods used in our investigations.

The hops experimental garden and hot-houses of the Carlsberg Laboratory were excellently suited for the pollination experiments which form the basis of the cytological investigations. These were carried out for the most part on microtome series of fixed material. To be able to follow the growth of the pollen tube through the tissues, the fertilization, development of the germ etc. the inflorescences of the female plants were artificially pollinated, so that it was known at any time how long a period had passed since the pollination. It was necessary naturally, always to isolate the female inflorescences in question in the young condition, to prevent natural pollination from the male plants growing in the experimental garden. For this purpose a number of the tips of the experimental plants were bound round by water-tight paper bags, which proved not to hinder the normal development of the hops or the male flowers in any way, unless the plants -- as sometimes happened — suffered from aphids; these increased in such cases with great partiality in the bags, the young being unable to get away. When the female plants were considered to be ripe for pollination, i. e. the styles large and sticky, the hops pollen was collected either in the experimental garden or from wild-growing plants, the bags removed and a soft brush used to dust the pollen over the styles, after which the bags were again attached and marked with the date. After this samples of the dusted heads were selected daily or sometimes more, sometimes less frequently, and the ovaries with their styles were carefully picked out with the forceps and fixed. —

In a similar way experiments were made on the vitality of the pollen, this after being kept for a shorter or longer time being placed on isolated, ripe female plants, observations being made thereafter as to whether fertilization took place or not.

The material however was collected not only in the experimental garden, but also on the wild growing places of the hops, thus in North Sealand (Faergelunden at Frederikssund), in Jutland (Marselisborg wood at Aarhus) and material for fixing was collected not only in the day time, but also at most hours of the twenty four, in order to overcome the difficulties which might arise from the nuclear divisions proceeding at certain times during the day and night. In Marselisborg wood, for example, the material was fixed at 8.30 p. m., 12 midnight and 4.30 a. m. Altogether 114 preparations were made, a great number of which however were of the nearest ally of the cultivated hops, *Humulus Japonicus*. About 25000 microtome sections have been examined under the microscope.

For the fixation of the hops flowers in the different stages various fixing media were experimentally employed, namely, weaker and stronger chrom-acetic acid solutions, the fluids of Carnoy, Bensley and Flemming, sublimate-glacial-acetic-alcohol, Juel's zinc chloride solution, platinochloride-osmium-acetic acid (after Hermann) and Merkel's solution. In the end I selected the three first-mentioned. The most certain results for the chromosome studies especially were generally obtained from Carnoy's solution, both for the older and younger flowers. The chrom-acetic acid solutions only succeeded in fixing the outermost cell layers and here acted irreproachably; deeper into the tissues, for example in the interior of young ovaries, on the other hand, shrinking occurred and the chromatine frequently appeared in clumps. The reason for this must be mainly, that the liquid penetrates so slowly, that the cells in the interval undergo pathological changes. Bensley's solution often gave excellent results, as it combined rapid penetration with an excellent power of fixation. Consequently, this — as well as that of Carnoy — was often used, when thicker tissues had to be fixed. By comparison with Carnoy's, Bensley's solution had the advantage of giving a softer and more pleasing fixation picture, quite free from plasmolytic phenomena.

For the staining of the objects many different methods were

used. Quite the best results were given, irrespective of the fixation, by Heidenhain's haematoxylin for the chromosome pictures and saffronin — anilin-blue (also gold-orange) for the tissues. The last combination can be recommended as excellent for most of the tissue sections and as a rule gave fine results, using the following procedure: 1) Treatment 1—3 hours or more with a solution of saffronin in 50 per cent. alcohol. 2) Rinsing in 50 per cent. alcohol. 3) Treatment 7—15 seconds, the object glass being kept in movement, with a solution of anilin-blue in 50 per cent. alcohol. 4) Differentiation in acid alcohol (3 drops diluted hydrochloric to 50 cm.^{3} 50 per cent. alcohol) 4—10 seconds, the object glass being kept in movement. 5) Rapid rinsing in 70 per cent., then in 90 per cent. and 95 per cent. alcohol. If after this the saffronin colour is too strong, the object is taken back to the acid alcohol; if it is too weak, it is placed again in the saffronin, after which the differentiating process can be repeated. On the other hand, if the anilin-blue is too strong it is difficult to save the preparation, if the blue colour has already been fixed in acid alcohol; the next preparation must then be stained for a shorter time. If the excessive staining is discovered before the acid treatment, the superfluous blue colour is washed off in alcohol before the fixing takes place. The blue anilin colour, however, is so very transparent, that even a strongly stained preparation shows as a rule good colour contrasts. Finally, the preparations may be tinged — after going through absolute alcohol — with gold-orange dissolved in clove oil, then washed in xylol and mounted in xylol-Canada balsam. A successful preparation shows sharp contrasts, the lignified parts and especially the chromosomes being a bright red, whilst the cellulose walls are blue and the cytoplasm, the epidermis and often the intermediate lamellae orange-coloured. Often, however, it is unnecessary to use any special plasma colour, as the cytoplasm is disposed to become faintly blue from the anilin-blue.

It may also be mentioned, that a number of experiments were made on the germinating power of pollen of *H. lupulus* and *H. Japonicus*. With regard to the methods used here reference may be made to section 7.

4. Formation of the embryo-sack.

Zinger's description (l. c.) of the formation of the embryo-sack in *Humulus* agrees fully with my observations, as regards the development of the nucellus, and I shall therefore cite the account of this author.

Während die Samenknospe noch ihre ursprüngliche Lage in der Basis des Fruchtknotens einnimmt, bevor die Integumente sich bilden, fällt es nicht schwer, im Gewebe des jungen Nucellus eine subepidermale Zelle zu unterscheiden, die auf der Längsaxe des Organs liegt und sich von den Nachbarzellen durch grössere Dimensionen auszeichnet. Nachdem das innere Integument der Samenknospe entstanden, seltener etwas später, nachdem die Anlage des äusseren Integuments bereits sichtbar ist, theilt sich diese Zelle in zwei ungleiche Tochterzellen: die äussere, kleinere, sog. Schicht- oder Tapetenzelle und die beinahe zweimal so grosse innere, die Embryosackmutterzelle. Gegen dieselbe Zeit, zuweilen auch vor der erwähnten Theilung oder bald darauf entstehen die Tangentialscheidewände in den Zellen der Epidermalschicht an der Spitze des Nucellus. Durch eine Tangentialwand theilt sich auch die Schichtzelle, so dass die Embryosackmutterzelle bald nach ihrer Entstehung gewöhnlich mit vier über einander gelagerten Zellen bedeckt wird. Die Mutterzelle selbst ist in diesem Entwickelungsstadium in die Länge ausgedehnt und enthält in ihrem vorderen Theile einen grossen, länglichen, an Chromatin armen Zellkern. Bald theilt sie sieh in zwei Zellen, deren die hintere, grössere, winderum in zwei Tochterzellen zerfällt. Die hintere von den drei auf diese Weise entstandenen Zellen ist der Embryosack. Während die beschriebenen, zur Bildung des Embryosacks führenden Theilungen noch vor sich gehen, vermehren sich sowohl diejenigen Zellen, die durch Theilung der Epidermalschicht entstanden sind, wie auch jene, die ihren Ursprung der Schichtzelle verdanken; diese und jene theilen sich hauptsächlich durch tangentiale Scheidewände und bilden insgesammt die massige Spitze des Nucellus der Samenknospe. Indem der Embryosack wächst und seine Geschwisterzellen wie auch die Zellen des benachbarten Gewebes verdrängt, dringt er nicht weit in das Gewebe der Kernspitze ein, so dass sein Vorderende zur Zeit der Befruchtung von vielen Zellenschichten, die, wie erwähnt, zweifacher Herkunft sind, verdeckt bleiben.

Was nun die Vorgänge im Innern des Embryosacks während seiner Entwickelung betrifft, d. h. die Theilung dessen Kernes, die Bildung des Eiapparats und der Antipoden, so zeigen die Abbildungen 4—8 Taf. X, welche die von mir beobachteten Hauptstadien vorstellen, dass diese Processe bei *Humulus* auf normale, den Angiospermen eigene Art verlaufen. Ueberhaupt bietet die Entstehung und Entwickelung des Embryosacks bei *Humulus*. wie wir sahen, weder Abweichungen, noch wesentliche Eigenthümligkeiten. Von Interesse scheint bei der Entwickelung des Nucellus der Samenknospe die Bildung einer vielsichtigen "Epidermalkappe" — eine Erscheinung, die bekanntlich bei den Angiospermen nicht oft zu beobachten ist."

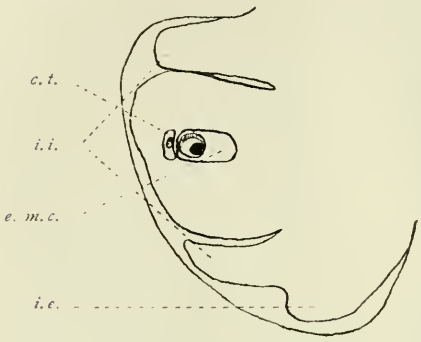


Fig. 1. *H. lup.* Median longitudinal section of a young ovule. One-celled archesporium and tapetal cell. *c. t.* tapetal cell; *e. m. c.* embryo-sack mother-cell; *i. e.* outer integument; *i. i.* inner integument. Apochr. 8 mm., comp. oc. 6).

Fig. 1 shows a section of a young ovary, in the ovule of which is seen a uninuclear archespore cell, which tangentially has cut off a tapetal cell out towards the tip of the nucellus. At this stage the inner integument only covers the sides of the nucellus. The tapetal cell is in general only covered by 2-3 overlying cells. Both these and the tapetal cell now divide actively by tangential and fewer radial walls. The archespore cell likewise divides into two daughter cells, both of which or only the innermost again divide, so that the ovule, when the inner integument is about to grow over the nucellus and the outer is already distinctly developed, contains a row of 3—4 megaspores (fig. 2), in which the innermost cell becomes the embryo-sack; out towards the tip of the nucellus this is covered by a tissue of about 8 cell-layers in thickness. The cells of the archespore and especially the young embryo-sack (fig. 3) elongates and become egg-shaped, and at the same time the whole ovule

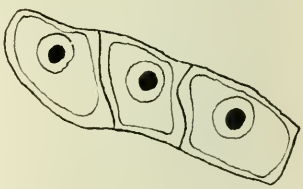


Fig. 2. *H. lup.* 3 megaspores. (Hom. imm. 2 mm., comp. oc. 6).

with the integuments becomes elongated. Of the last the inner has now gradually grown quite over the tip of the nucellus, whilst the outer has not quite succeeded in completely encompassing the inner, for which reason the apex of the nucellus is only covered by the inner integument (fig. 4). It is characteristic, as is also remarked by Zinger (l. c.), that the two integuments

Fig. 3. *H. lup.* Median longitudinal section of a young ovary. Ovule with young embryo sack. *i. e.* outer integument; *i. i.* inner integument; *nuc.* nucellus; *perig.* perigonium. (Apochr. 8 mm., comp. oc. 6; reduc. t. $^1/_2$).

grow together at the tip behind, so that here no sharp boundary between them can be observed. Zinger states (cf. citation p. 3), that the micropyle is quite wanting, and from this he draws other conclusions regarding the phylogeny of *Humulus*. He is strongly opposed to Lermer and Holzner's earlier statement (l. c. II) of the presence of a well-developed micropyle. In reality I have never observed any ripe ovule, in which the micropyle was quite wanting. This is always — though often feebly — developed, but I must assume that Lermer and Holzner's account is

somewhat exaggerated. The opening of the micropyle towards the cavity of the ovary is as a rule narrow, but the lumen becomes wider down towards the nucellus (fig. 5). At this stage it is seen, that the ovule from being straight with the apical

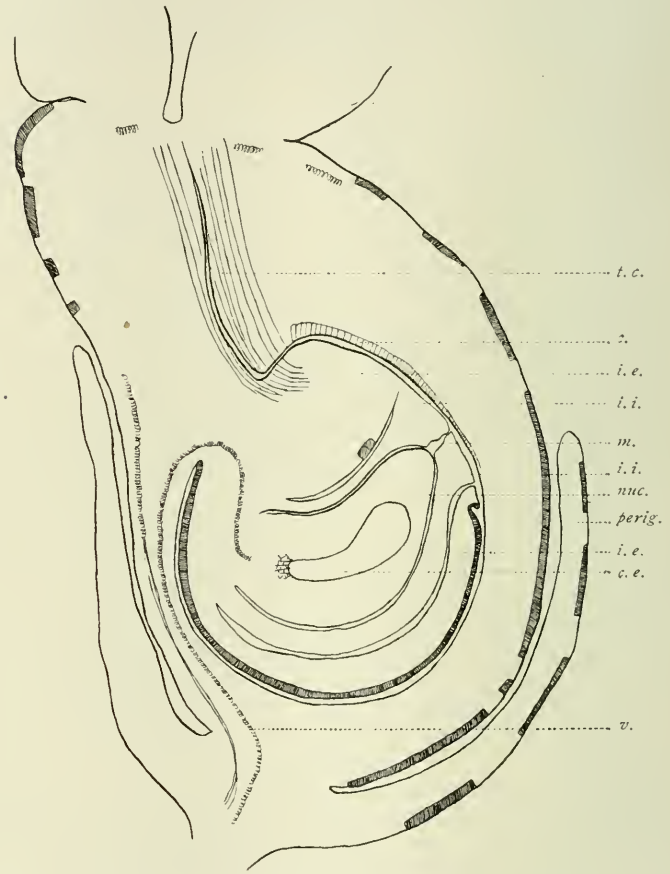


Fig. 4. *H. lup.* Median longitudinal section of almost ripe ovary. *c.* innermost cell-layer of the ovarial wall; *c. e.* embryo-sack; *i. e.* outer integument; *i. i.* inner integument; *m.* micropyle; *nuc.* nucellus; *perig.* perigonium; *t. c.* conducting tissue; *v.* vascular bundle. (Apochr. 8 mm., comp. oc. 4; reduc. t. $^1/_2$).

end pointing obliquely downwards has begun to bend slightly, the apex tending to turn upwards (fig. 4). At this point the embryo-sack contains 8 nuclei due to successive nuclear divisions, and 4 nuclei lie together at each end of the sack (fig. 6). In the course of time the whole ovule bends more and more, so that the embryo-sack at the stage of fertilization already forms

a curve corresponding to $^1/_3$—$^1/_2$ of a circle (fig. 7); the distal end of the embryo-sack now contains much more space than the proximal, being swollen like a club. Downwards the lumen of the embryo-sack decreases greatly in width, but again at the very base expands slightly (fig. 7). When the embryo is in process of development, the ovule is so bent, that the micropylar region lies close up to the chalaza. — The nuclear phenomena however have proceeded normally, one nucleus from each pole of the embryo-sack having fused to form the central nucleus (fig. 8, 9). Usually the egg-nucleus and the central nucleus are the only well-developed and apparent nuclei in the mature embryo-sack; as the rule the synergids soon disappear and the three small antipodals, which are situated in the slightly expanded, proximal end of the embryo-sack, gradually shrivel up. The

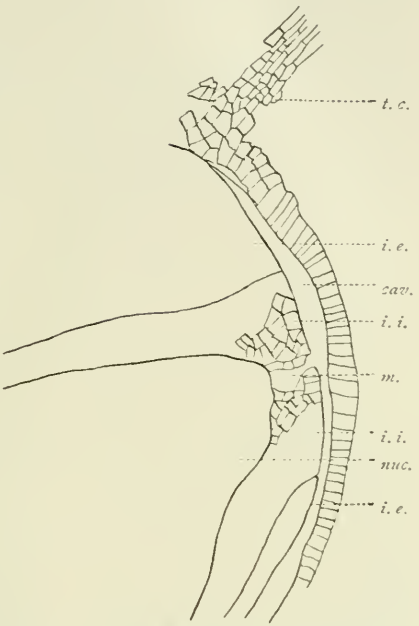


Fig. 5. *H. lup.* Micropylar region of a ripe ovule. *cav.* ovarial cavity; *i. e.* outer integument; *i. i.* inner integument; *m.* micropyle; *nuc.* nucellus; *t. c.* conducting tissue. (Apochr. 8 mm., comp. oc. 4; reduc. t. $^1/_2$).

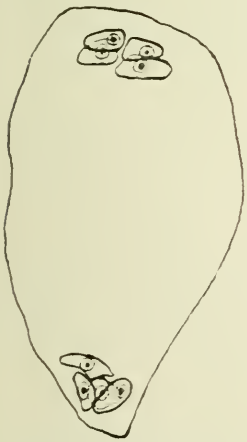


Fig. 6. *H. lup.* Young embryo-sack with 8 cell-nuclei. (Apochr. 3 mm., comp. oc. 6).

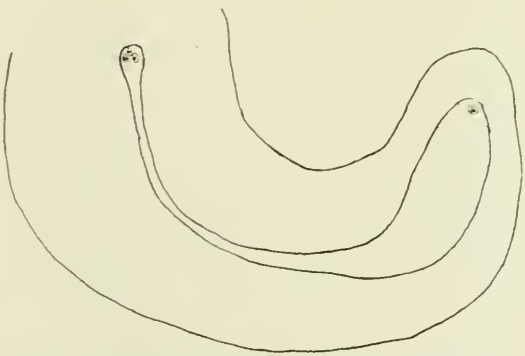


Fig. 7. *H. lup.* Nucellus with mature embryo-sack. (Apochr. 8 mm., comp. oc. 4; reduc. t. $^1/_2$).

central nucleus always lies nearest the concave side of the embryo-sack which is turned towards the apex of the ovary (fig. 9).

In *H. Japonicus* the development of the ovule proceeds in quite the same manner. The essential difference consists in this, that the ovule is bent up earlier and more strongly, and that the embryo-sack at the stage of fertilization is still longer and thinner than in *H. lupulus*. As in the latter the apical end is greatly swollen, whilst downwards the embryo-sack is very thin,

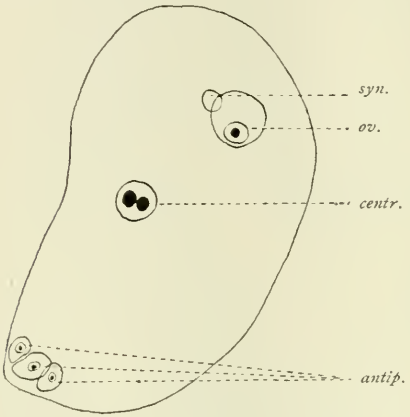


Fig. 8. *H. lup.* Fusion of the polar nuclei. *antip.* antipodals; *centr.* central nucleus; *ov.* egg-cell; *syn.* synergid. (Apochr. 8 mm., comp. oc. 6; reduc. t. $^2/_3$).

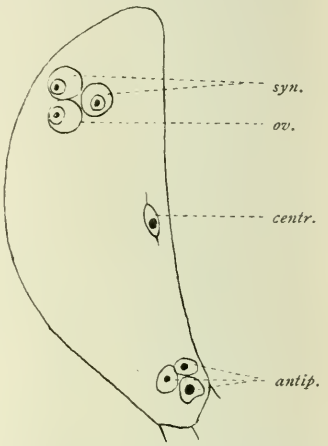


Fig. 9. *H. lup.* Central nucleus fully formed. *antip.* antipodal cells; *centr.* central nucleus; *ov.* egg-cell; *syn.* synergids. (Apochr. 8 mm., comp. oc. 6; reduc. t. $^2/_3$).

worm-shaped and very little or not at all wider at the base (fig. 10). During the development of the embryo the embryo-sack forms an even greater arc than in the common hops, and the micropylar region lies extremely close to the chalaza. But in addition there is another characteristic difference in the structure of the ovule in the two species, the inner integument in the Japanese hops never growing over the tip of the nucellus, which thus always stands in free connection with the ovarial cavity (fig. 10).

In both species of hops there is a characteristic parenchyma (hypoblast) with solid cell-walls in the nucellus at the base of the embryosack. These have already been referred to by Westermaier (1890); as mentioned by Lermer and Holzner they show a strong preference for anilin colours and are not dissolved in

sulphuric acid. Modilewsky (l. c.) also mentions this tissue in the Urticiflorae and connects it with the nourishment of the embryo-sack. In *H. Japonicus* crystals of calcium oxalate are specially common in the ovary, especially in the tissue lying over the ovule — but not in the conductive tissue.

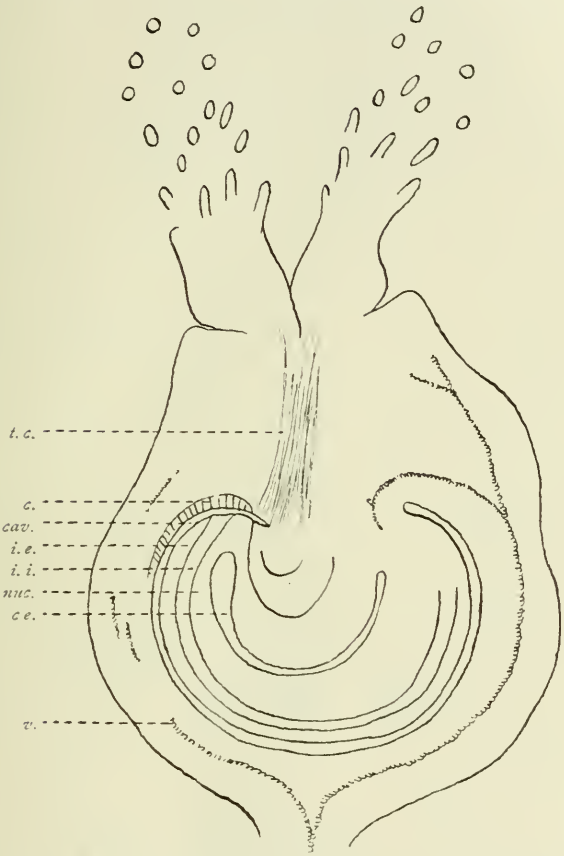


Fig. 10. *H. Jap.* Median longitudinal section of ripe ovary. *c.* innermost cell-layer of the ovarial wall; *cav.* ovarial cavity; *c. e.* embryo-sack; *i. e.* outer integument; *i. i.* inner integument; *nuc.* nucellus; *t. c.* conducting tissue; *v.* vascular bundle.

5. Formation of the pollen.

In the young anthers we see the four sporogenous tissues which mark off the archespore in the four, original pollen sacs and which outwardly are only bounded by a wall of two cells in thickness. The outermost of these two cell-layers, the epidermis, only undergoes anticlinal divisions, whereas the subepidermal

layer, which is the sister-tissue to the archespore mother-cells, forms by periclinal divisions the wall 4—5 cells in thickness, which as the anthers become larger can be seen between the spore mother-cells and the epidermis (figs. 11—12). The course

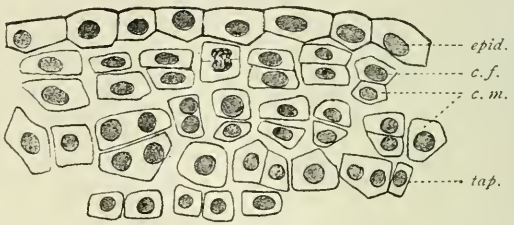


Fig. 11. *H. lup.* Part of transverse section of young anther. *c.f.* the fibrous cell-layer; *c.m.* intermediate layer: *epid.* epidermis; *tap.* tapetum.

of these divisions is, that the subepidermal cell-layer divides by periclinal walls, two layers thus arising of which the innermost is the tapetum. The subepidermal cell-layer again divides periclinally into two cell-layers, of which the one or both in the end

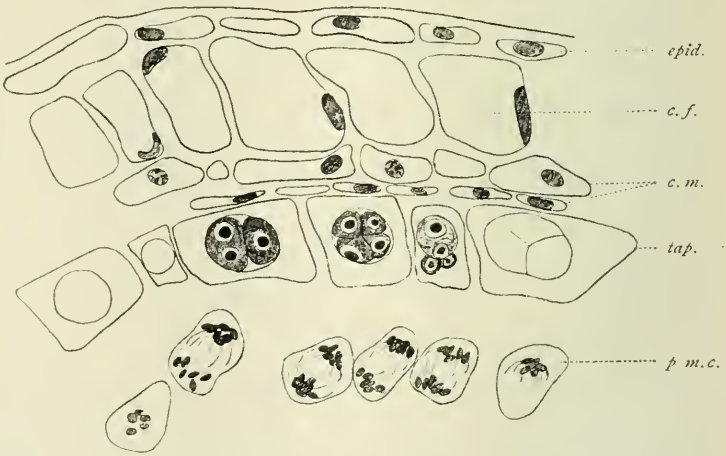


Fig. 12. *H. lup.* Part of transverse section of anther. *c.f.* fibrous cell-layer; *c.m.* intermediate layer; *epid.* epidermis; *p.m.c.* pollen mother-cell; *tap.* tapetum.

undergo a further periclinal division. The intermediate layer between the tapetum and subepidermis (the fibrous layer) thus consists of 2—3 cell-layers. These are formed of greatly flattened and small cells, specially small in the innermost layer nearest the tapetum (fig. 12).

The cells of the archespore divide up repeatedly as usual

by bipartition and the nuclear divisions are always completed long before the cell divisions are begun, so that binuclear elements are very common. The divisions give rise to the formation of spore mother-cells, which after the usual tetrad division result in the pollen grains. These have no sculpture, are spherical and provided with three germination pores and are ca. 19—23 μ in diameter.

In *H. Japonicus* the conditions are essentially the same. The pollen grains are formed under similar circumstances and are very little different from those of *H. lupulus*. In the fresh state they can be distinguished from those of *H. lupulus* by having a more hyaline, uniform plasm, whilst the pollen grain of the common hops has denser, more turbid contents.

The growth and further development of the pollen grain is described in section 7.

6. Special nuclear conditions.

In studying the finer nuclear conditions in the hops I fixed several of the organs to determine, which tissue gave the best figures of the nuclear divisions. It proved, that the somatic nucleus could be best observed in the tapetal cells of the anthers, the archespore mother-cells and in the ovarial wall; on the other hand the ends of the root were unsuitable, though the nuclei here were often in active division, as the ends of the roots in *Humulus* are extremely thin, scarcely over $^3/_4$ mm. in diameter, the cells small and also more especially the chromosomes. The nuclear spindle however was more clearly formed in the bark cells of the tip of the root than anywhere else. Nor were the tips of the stems suitable, which is undoubtedly connected with the fact, that the young tissues here are exceedingly rich in tannic acid and that the cells are fairly small. It might be imagined, that the bracts of the hops would be well suited to the investigation of the division-phases of the somatic nucleus, since very active growth is in progress here when fertilization has taken place and the hops begin to ripen; but this did not prove to be the case either, the presence of chloroplasts and difficulties with the fixing causing too much indistinctness in the pictures.

The structure of the gonotokont nucleus was studied almost

exclusively in the gonotokonts of the male plant, as a study of the female plant in this regard offers great difficulties owing to the single ovules and their curved structure as well as the form of the inflorescence. Even when a very large number of young ovules with attached styles were picked out and after fixing embedded in paraffin in parallel position, it proved impossible to follow here the progress of the reduction divisions.

Both species of hops were examined. *H. Japonicus* as a rule proved more convenient than *H. lupulus*. the former as annual plant completing the whole of its ontogeny much more quickly whilst its cells and chromosomes are larger and fewer than in the common, perennial hops. As the two species are similar, they are taken together in the following.

The somatic nucleus. It is not difficult to determine, that the nuclei of the hops are of quite the normal structure. At the base of the styles we find a tissue, which is extremely well suited to the observation of the resting, somatic nucleus, though nuclei, cytoplasm and cell-walls have here a somewhat more ephemeral structure than is typical for the other tissues of the hops plant. I have found, that the number of chromosomes in *H. lupulus* is 20, in *H. Japonicus* 16 in the somatic cells.

It would be of interest to find some satisfactory explanation of the peculiar condition in both the plant and animal world, that by far the greater number of species have a number of chromosomes which is divisible with 4, very often indeed with 8, in the 2 x generation, which indicates that a doubling of the number of chromosomes frequently occurs on the formation of a species.

Fig. 23 shows a cell from the wall of the ovary of *H. lupulus*. in which 20 very long chromosomes are seen in the nuclear plate, and fig. 24 an anaphase in an archespore mother-cell in *H. Japonicus*. The chromosomes in fig. 23 have quite a different form from those in the gonotokont nuclei, where they are always very short; and even in the other somatic cells the chromosomes are seldom so long as in the case figured. No individual differences could be noticed externally in the chromosomes of *Humulus:* they are all very much alike, but this naturally does not prove, that they are wanting in individual characteristics internally.

The tapetal cells of the anthers are of some interest, as they

give an excellent example of vegetative nuclear fusions. It is well-known, that the tapetal cells often become bi- to multinucleate, but we seldom, I imagine, find this so well-marked as in *Humulus,* and the condition may therefore be described more closely.

In recent years a theory has appeared regarding the phylogeny of the tapetal cells, namely, that they should be considered as sterile pollen mother-cells. This point will be considered more closely in the following and I may at once remark, that my investigations bring me into conflict with the theory put forward.

From the beginning the tapetal cells are not specially different from the neighbouring cells externally (fig. 11) or from the mother-cells of the archespore, but when the pollen mother-cells are formed and the reduction division begin in these, the tapetal cells have reached quite a considerable size and appear in section as rectangular, radially extended cells with chromophile cytoplasm. The nuclei and also the chromosomes are large, the latter being present in the number normal for somatic cells. As a rule the nuclei divide up mitotically, when the gonotokont nuclei are in synapsis, thus giving rise to binucleate tapetal cells (fig. 25), but often the two nuclei fuse together again without the chromosomes uniting, thus producing a divalent nucleus with 4 x chromosomes.

In fig. 26 is shown a peculiar diakinesis-like prophase in *H. Japonicus* in two sections, which has only been noticed this once; 16 (17?) divided chromosomes can be observed. The picture calls to mind a tetrad division in which only the chromosomes, but not the nuclei themselves are dividing (comp. fig. 46). I suppose that it is a case of abnormal, indirect vegetative reduction like those of Němec (1910). — Before the fusion takes place, another division sometimes occurs synchronously in the two nuclei, after which the products of division again unite, — and thus quadridivalent nuclei are formed. More frequently the two divalent nuclei unite and thereafter undergo a division in which 4 x chromosomes are present. It is impossible to count exactly the large number of chromosomes which arise in this way, but certain pictures, e. g. fig. 27, indicate, that nuclei with even greater valency may occur. This figure shows a tapetal cell of *H. Japonicus*, in which two didivalent nuclei are in prophase, a couple of chromosomes showing distinct longitudinal splitting

for the following division. Thus octodivalency must occur after this division. The fusion of the resting daughter-nuclei in the tapetal cells occurs essentially in the manner decribed by Bonnet (1912); yet I have not been able to observe, that any special affinity between the nuclei is apparent in the distribution of the chromatin in these. Bonnet states (l. c.) that this polarisation shows itself in a "concentration de la chromatine le long des faces aplaties des noyaux" (p. 651), an observation which is in agreement with Rosenberg's description (1901, I) of the endosperm nuclei in *Zostera marina*. — In *Humulus* I have never observed four, but at most two, free-lying resting nuclei in the tapetal cells. If four nuclei are present, they unite already very early or are seen lying as a synkaryon. Fig. 13 shows two neighbouring tapetal cells each with its synkaryon of uni- and plurividalent nuclei. It remains uncertain, however, whether in the end it is also the actual single or double nuclei which are individualised with appertaining chromosomes, or whether it is not a question rather of chance concretions caused by the chromosomes, more or fewer in number, grouping themselves each round its nucleolus, to which they gradually give up the chromatin substance. Fig. 28 shows the typical form of the chromosomes of the tapetal cells. These very often are short, rod-shaped with sharply truncated ends. When the tapetal cells become older, both the cytoplasm and nuclei lose their chromophile character. In the nuclei the linin threads can be seen, to which the chromosomes are apparently attached (fig. 29). These latter are dissolved as the nucleoli develop. Lastly, when degeneration sets in, the nucleoli also become smaller. At the stage when the plurivalent nuclei have come to rest and begin to degenerate, the tetrad division is complete and the pollen grains as a rule fully formed.

Regarding the general significance of these phenomena in the tapetal cells Bonnet remarks in his summary (l. c. p. 712): "Les cellules nourricières paraissent être des cellules archesporiales redevenues végétatives. Elles offrent des analogies complètes avec les microsporocytes abortifs des hybrides stériles." It was Rosenberg (1899) who put forward this view to which Bonnet, in spite of a few differences between the authors, gives his support. Various arguments have been put forward in defence of the view, that the tapetum consists of somatised pollen mother-

cells, especially these (Bonnet l. c.): 4 nuclei are often formed in each cell and the last division occurs synchronously for both daughter-nuclei and further almost simultaneously the mitosis of the spore mother-cells.

Bonnet himself remarks in this connection however, that the literature contains statements of various irregularities both in the pollen mother-cells in several plants and in the tapetal cells. Thus, in certain plants only 2 microspores are sometimes formed from each microsporocyte *(Convallaria multiflora, Aconitum*

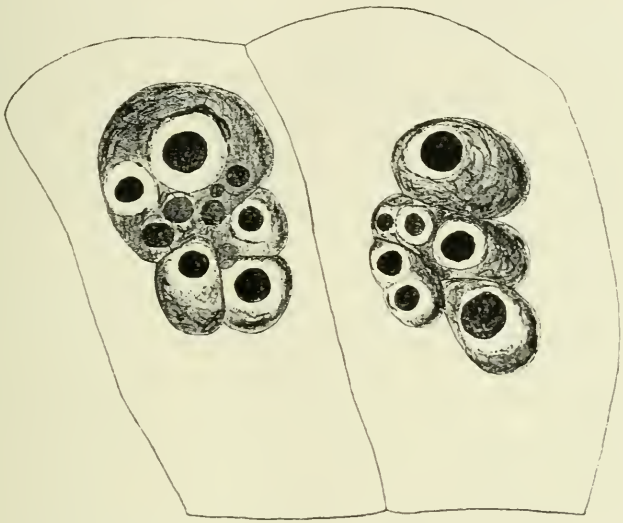


Fig. 13. *H. Jap.* Tapetal cells of anther with synkaryon-like nuclear concretions. (Hom. imm. 2 mm., comp. oc. 18; reduc. t. $^2/_3$).

Napellus, Asparagus officinalis), and sometimes there is only one nucleus in each fully ripe tapetal cell *(Helleborus viridis)*, sometimes 2 *(Adoxa, Silphium, Nymphaea alba)*, sometimes 4 *(Yucca, Fuchsia, Atropa Belladonna, Drosera)* and sometimes more *(Ribes intermedium, Mirabilis Jalapa, M. tubiflora, Hepatica)*. Bonnet likewise remarks briefly, that synchronous divisions occur many other places than in the microsporocytes, thus usually in all multinucleate cells, when these are not specially large. On the other hand, endosperm nuclei, nuclei of laticiferous vessels and nuclei of giant cells often divide asynchronously — though examples of the contrary are also known — but pass through the caryokinetic division in such a way, that the nuclei placed diametrically opposite each other in the cells represent respectively

the least and most advanced stage, whilst the remainder show all the intermediate stages in well-arranged series. Bonnet explains further, that he and several others constantly find the tapetal nuclei in division earlier than the microsporocytes. All these facts weaken to no little degree the value of the evidence for the atavistic characteristics of the tapetal cells.

As mentioned above, however, Bonnet adheres to this view and I shall cite the following (l. c. p. 707), which for Bonnet obviously contains decisive evidence of the correctness of Rosenberg's hypothesis.

"Enfin une dernière remarque que je ferai au sujet de ces mitoses est qu'elles ne possèdent pas du tout, dans l'ensemble des diverses cellules-nourricières d'un même sac pollinique, ni même à un niveau donné de ce sac, le synchronisme si remarquable qui est une loi générale pour les cellules-mères du pollen. Tandis que, dans un sac pollinique donné, celles-ci sont toujours toutes en synapsis, ou toutes à la métaphase I, ou toutes à l'anaphase II, par exemple, on observe au même instant et dans la même coupe des cellules-tapètes à 1, 2 ou 4 noyaux, des noyaux en division, d'autres au repos, etc.

Mais à ce propos il faut signaler deux observations très remarquables:

1. Gates (1907), signale, que, chez *Oenothera lata*, "this first nuclear division in the tapetal cells may take place almost simultaneously throughout a loculus, so that many mitotic figures in various stages are often found in the same section" (p. 85).

2. Tischler (1906), dans l'hybride *Bryonia alba* ♀ × *Bryonia dioica* ♂, observe (note de la p. 86), que les divisions des sporocytes ne s'effectuent plus synchroniquement dans un même sac pollinique. "So konnten selbst noch Kerne der Pollenmutterzellen gefunden werden, die sich erst zur I. Mitose anschickten, während andre schon beide Tetradenteilungen vollendet hatten."

Et parallèlement cet hybride était stérile. Le rapprochement de ces deux observations, sourtout si on les compare avec ce que j'ai déjà dit sur les hybrides végétaux stériles et les irregularités de leur microsporogénèse, ne laisse pas que d'être suggestif."

Bonnet's remark, that divisions of the pollen mother cells always take place simultaneously throughout the whole sac, regarding which he only knows the statement cited of Tischler

for the hybrid *Bryonia*, is not justified, as *Humulus* shows. The condition here is this, that the nuclear divisions take place basipetally in such a manner, that for example the base of a pollen sac contains nuclei in synapsis, whilst nuclei near the apex have already completed the heterotypical division and lie in a kind of resting stage, during which the nuclei are slightly chromophilous, before the beginning of the homotypical division. The tapetal cells here moreover behave in a corresponding manner, as they follow a similar, though less regular development basipetally.

Comparison with sterile pollen in hybrid plants does not seem to me on the whole to give important resemblances. Some agreement must naturally always be present between tapetal cells and hybrid pollen mother-cells, both of which lead a very short life and degenerate early. The time for the divisions in the pollen mother-cells and the later maturation of the pollen undoubtedly depend a great deal on the structure of the anthers. In the hops the pollen sacs open by an apical slit, and the apical pollen grains must naturally also fall out before the basal. There is scarcely any doubt, that the ripening of the pollen grains basipetally is connected with this fact.

A more important support seems to be, that Rosenberg from an investigation of *Zostera marina* (1901, II) finds, that tapetal cells are cut off from the cells of the archespore directly, a condition that would be of interest to verify by more observations — and by a study of related plants.

Personally I doubt that the origin of the tapetal cells from somatised pollen mother-cells is a generally valid, phylogenetic rule and I imagine, that the characteristic cytological features of the tapetal cells are closely connected with the physiological requirements placed upon this tissue and have nothing to do with the tetrad division of the pollen mother-cells. So far as I can see, there is only this resemblance between tapetal cells and the pollen mother-cells of sterile hybrids, that both may often be binucleate. Since now in addition the two nuclei of the tapetal cells arise in the ordinary homoeotypical manner and thus have 2 x chromosomes, whilst the pollen mother-cells by heterotypical division obtain two univalent nuclei (with x chromosomes), it became necessary to assume, that during the phylogeny a further, inner change — a somatisation — had taken place in the tapetal

cells, a theory that does not seem to me acceptable without further investigation.

It seems to me much more probable, as mentioned, that we can find a more physiological explanation in the characteristic, cytological conditions of the tapetal cells. — The first point here is, that tapetal cells are characterised by having 2 or more nuclei formed in them by repeated mitotic division of one original nucleus. These nuclei are not distributed round about in the cytoplasm of the cell, but display mutual affinity and finally unite; they thus act here neither like the 2 or 4 nuclei in pollen mother-cells nor like nuclei in normal, multinuclear, somatic cells, which are always distributed fairly evenly in the cytoplasm. The fusions give rise to a larger or smaller synkaryon-like key of more or fewer united nuclei, a conglomerate very rich in chromosomes. We may suppose, that it is of practical value for the plant, that the tapetal cells have a large supply of chromosomes, the intensity of the metabolic and anabolic processes being thus increased, as the cells gain in capacity from the rich supply of nuclei. In the case of the tapetal cells it is evident, that these during the development of the archespore to pollen grain must be the exclusive agent in conveying the necessary nutriment to this tissue and it thus seems only reasonable to conclude, that the tapetum possesses special qualities to attract the nutriment materials necessary for the pollen mother-cells. In support of this explanation we have the fact, that the tapetal cells are already as a rule hyperchromatic, often with didivalent or quadridivalent number of chromosomes, at the end of the synapsis stage, when the actual transference of chromosomes and similar, energy requiring processes are about to begin in the pollen mother-cells.

We might well compare the condition of the tapetal cells with that of the endosperm, since the latter has a similar function to the tapetal cells. Just as the tapetal cells provide the pollen mother-cells with nutriment, it is the function of the endosperm to nourish the germ. It is striking therefore, that the nuclei of the endosperm are also hyperchromatic normally, though not to so great a degree as the tapetal cells. Certainly the large number of chromosomes is not reached here by simple mitotic division and subsequent fusions, but by the cooperation of the male sexual cell. By the normal fusion of a synergidal and an antipodal polar nucleus the central nucleus obtains 2 x chromo-

somes and by double fertilization 3 x chromosomes. It seems to me a very important fact — all the more that there is no other satisfactory explanation of the phylogenetic origin of the endosperm — that the only two places in the ontogeny of the plant, where more than 2 x chromosomes are normally present in the nucleus, are in cells which to a high degree are connected with the function of nutriment. This indicates, that the rich supply of chromosomes in the endosperm and tapetum must be explained from purely physiological considerations. That a male nucleus takes part in the embryo sack in the formation of the endosperm, is connected, I believe — as is also pointed out by Němec (l. c.) — with the nourishment of the germ, since the endosperm as a product of the two sexes must be considered to be a more natural means of nourishment for the germ than a nutriment tissue formed without the cooperation of both sexes, and — we might add — especially where it is a question of plants which require foreign pollination, especially also dioecious plants.

It is also well-known, moreover, that large nuclei and with them large chromosomes usually occur in tissues, which are distinctly connected with nutrition, e. g. in the suspensor.

Rosenberg (1904) states: "Es scheint mir berechtigt, den Chromosomen eine aktive Tätigkeit an der Umbildung der "Nahrungsstoffe" zuzugestehen. Mit Sicherheit ist eine Korrelation zwischen der Nahrungsarbeit der Zelle und der chromatischen Substanz des Kerns nachgewiesen worden" (p. 258).

Němec (1910) writes: "Bekanntlich erscheinen grosse Chromatinansammlungen in Kernen, welche sich in Zellen befinden, die eine rege Stoffwechseltätigkeit entwickeln" (p. 388).

Several examples of hyperchromatic cells are known in addition to the just mentioned tapetal cells and endosperm, but these are nevertheless the commonest examples among phanerogamic plants. It should be mentioned, that Chamberlain (1909) figures binuclear microsporangium tapetal cells in *Dioon edule*.

In a number of plants there occurs with greater or less regularity a fusion of nuclei in the endosperm, either of sister-nuclei (*Secale cereale*) or of more distantly related nuclei (*Corydallis cava*) (Strasburger, 1880). As mentioned above, Rosenberg (1901, I) finds similar conditions in *Zostera marina*. It may remain unsaid, whether this vegetative caryogamy has the

same causes as the nuclear fusions of the tapetum, — and the same applies to the hyperchromatic qualities of the antipode nuclei in Lilium Martagons and the embryo-sack of other monocotyledons observed by Guignard (1891). Of greater interest in this connection is Dixon's statement regarding *Pinus silvestris* (1894), that the gametophyte has 8 chromosomes in the young endosperm, but that the larger cells of the archegonial wall, which in appearance and probably also in function greatly resemble the tapetum of the anthers, nevertheless usually have 12—24. In addition we have another, very important fact, namely this, that cells in the archegonial wall in the Cycads may show synkaryon-like products from nuclear fusions, which quite resemble those occurring in the tapetum. Thus in material of *Ceratozamia?*, which Dr. Chamberlain most kindly lent me in 1911 in Chicago, I have observed figures like that given in fig. 14, which show typical, hyperchromatic archegonium tapetal cells. It goes without saying, that such cells, which belong to the gametophyte, are not hyperchromatic owing to atavistic tendencies, of which we should be able to find some explanation in the phylogeny.

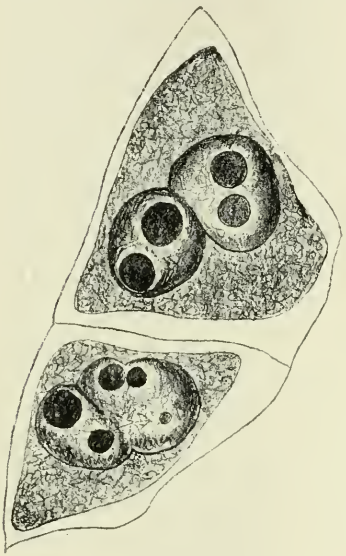


Fig. 14. Cycad *(Ceratozamia?)*. Two archegonial tapetal cells with synkaryon-like nuclear concretions. (Apochr. 3 mm., comp. oc. 6; reduc. t. $^2/_3$.

Such examples as are referred to above can only support the theory put forward, since in all cases — even the most remote — we have just to deal with cells, whose main function is to supply nourishment to others. It would be of interest to examine the characteristic tapetal layer, which in certain phanerogams bounds the embryo-sack, for this layer is without doubt connected with the nutrition. Warming (1913) mentions and figures, among others, *Escallonia macrantha*, where "le tégument devient très épais et sa couche interne se forme en épithélium" (p. 33). It is probable, that the cells of such tissues will also prove to be often hyperchromatic.

I thus believe, that the tapetal cells of the anthers are not metamorphosed, somatised archespore cells and that this consequently cannot be the explanation of the characteristic, cytological phenomena peculiar to them, but that the tapetum is a special tissue developed in connection with the nutrition — specially through the abundance of chromatin substance which is usually found here.

The gonotokont nucleus. In *Humulus lupulus* under our climatic conditions there passes about a month as a rule between the stage when the dense male inflorescences are just apparent until the pollen grains are set free. The greater part of this interval is taken up in the formation of spore mother-cells and, as mentioned previously, we find in the youngest anthers numerous binuclear elements in the archespore as also here and there mitotic nuclear division pictures. The young spore mother-cells are then seen for a long time to be in prosynapsis and synapsis, and the tapetal cells, which at first greatly resemble the gonotokonts, begin to appear during this last stage with 2 nuclei, as mentioned in the previous section. I have not had the opportunity to observe evident pairing of the prochromosomes in prosynapsis. The gonotokonts are in prosynapsis very small, but grow considerably in the synapsis stage, as also their nuclei, while the chromatin bundle becomes lighter (figs. 30 and 31). During this stage the doubling of the chromatin threads is seen and at a little more advanced stage this doubling can often be observed in a special manner, as shown in fig. 32, where a number of well separated, long chromatin threads are bent into the U shape. I must suppose, that it is the original doubling from (prosynapsis and) synapsis, which is constantly in evidence here, that, in other words, the somatic chromosomes 2 and 2 have fused to form the U (seldom the O shape) in such a manner, that each branch of the U represents an original, somatic chromosome, yet in such a way that certain exchanges must be assumed to have taken place between the chromosomes lying together in synapsis. After this the chromatin threads shorten and form chromosomes, which constantly reveal their divalent nature by being split longitudinally and only fusing together at the one or both ends (figs. 33, 34). In *H. lupulus* and *H. Japonicus* we find respectively 10 and 8 ring-like, double chromo-

somes shaped like a figure of 8 or almost entirely divided (fig. 35). Later in the prophase we often see only U or V shaped figures (fig. 36). In the following division the parts of the double chromosomes are separated from each other (fig. 37) and the half part withdraws each to its own pole with great regularity (figs. 38, 39, 40, 41).

It may be emphasized, that in *Humulus*. as the foregoing shows, I find, that the parallel position 2 and 2 of the chromosomes in diakinesis originates already from synapsis. Presumably it is the parent chromosomes in the somatic cells, which are always individualised.

Just as no sex-determining idiochromosome has hitherto been found in any dioecious plant, there is just as little any sign of this in the microspores of *Humulus*. In the anaphase of the reduction division I have several times succeeded in determining the number of chromosomes at both poles, as both groups of daughter chromosomes could be observed in nuclei lying a little obliquely. Such nuclear figures are shown in figs. 40 and 41. In many cases 10 and 8 chromosomes could be observed in a few nuclear plates in the anaphase (figs. 42, 43, 44). When the daughter-nuclei have reached the telophase (fig. 45), the splitting for the next division can often be seen already in the chromosomes. This splitting follows soon afterwards (figs. 46, 47, 48, 49) and the young spore tetrads appear (figs. 50, 51). The single chromosomes can still be seen in the newly formed pollen grains for a short time, after which the nucleus passes into the resting stage. Later the first generative division occurs (fig. 52).

The tetrad division and maturation of the pollen grains, as mentioned above p. 20, takes place basipetally. On maturation the flower opens, the filaments are very rapidly elongated and the pollen grains are set free through the apical pores of the pollen sacs.

A couple of abnormal cases in the formation of the microsporangia and their further development, which do not seem without interest, may still be mentioned.

1. In July 1912 the Laboratory gardener Chr. Smith observed in our experimental garden, that a wild hops taken from the woods and planted there bore a number of male flowers in addition to the normal female inflorescences. Since then several

cases of monoecious plants have been found in the garden. Without going too deeply into the ramification it may be mentioned, that the male flowers formed as a rule small inflorescences with few flowers. which composed the lowest shoots on otherwise hop-bearing branches. Sometimes female elements (carpels) could be noticed in these extraordinary male flowers, thus one flower might have 3 anthers and 1 carpel or 3 anthers and 2 carpels, sometimes they were apparently quite normal. These monoecious plants were distinctly protogynous; it even proved that in the separate, bisexual flowers the styles were mature, i. e. developed and sticky, whilst the anthers still lay concealed inside the flower, from the tip of which the styles thus projected. The male flowers however died off fairly early and a cytological investigation of the conditions showed, that pollen mother-cells certainly were formed and that an apparently normal reduction division took place in these, but that they thereafter shrivelled up without even making the tetrad division (figs. 53, 54). On following the changes in the tapetum, for example, it became apparent, that the cells here instead of dividing up by radial walls as usual, showed no divisions at all. Nevertheless the outer cell-layers grew and thus the whole pollen sac. The result of this was, that in older anthers it was possible to observe a tapetal cell layer, in which the single cells were separated — ruptured — and unable to enclose the archespore, whose contents of pollen mother-cells likewise had assumed an abnormal appearance, the single gonotokonts lying scattered in the pollen sac and not forming a densely packed whole as in normal anthers. In *Oenothera* mutants and bastards Gates has found, that pollen mother-cells and tapetum develop in a fairly similar manner; for example, in *Oenothera rubrinervis* (1908) and *O. lata* (1907). The explanation of these phenomena must probably be sought for in the abnormal plant's inner physiology, which in spite of its bisexual nature is not sufficiently adapted to the nourishment of both the male and female flowers at the right time. No nourishment is brought to the tapetum and the cells gradually languish without being able to satisfy the large demands of the pollen mother cells lying inside. Thus, I consider it connected with peculiarities in metabolic regulation, when we do not find both micro- and macrospores in such a monoecious hops plant, and not dependent on fundamental laws as regards the sexual differences. On the contrary,

I think it probable, that a monoecious hops plant under suitable conditions or the right stimuli would be able to show normal spores of both kinds.

2. Among some hops plants from the United States, which flowered for the first time in the year 1913, an extremely peculiar specimen was found, which towards the summer became distinguished by its abnormal appearance. In spite of the fact, that no female flowers occurred on it, it mostly resembled a female hops plant. Examination showed, that the flower-bearing bran-

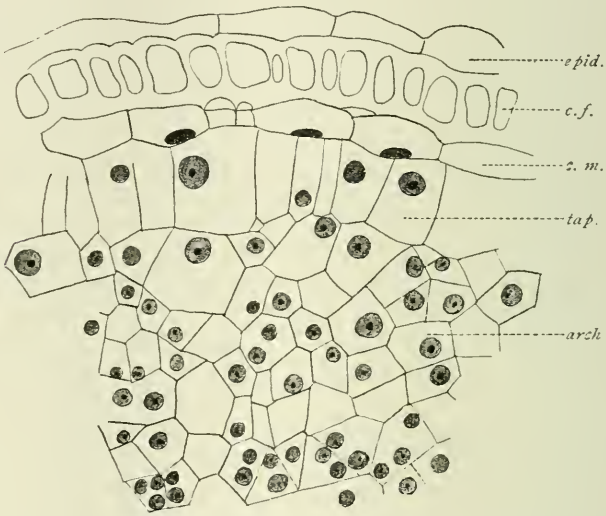


Fig. 15. *H. lup.* Gynomorphous male. Part of transverse section of aposporous anther, filled with a parenchyma with in part multinuclear cells, *ark.* the sterile archesporium; *c. f.* the fibrous cell-layer; *c. m.* intermediate layer; *epid.* epidermis; *tap.* tapetum.

ches bore small, few-leaved, somewhat dwarfed cups, which had male instead of female flowers. These male inflorescences were richly beset with lupulin glands, but the single flowers often looked fairly normal, as all five anthers with surrounding flower could be developed. A cytological examination showed, that in the pollen sacs no pollen mother-cells at all were formed; instead of the archespore there was a loose parenchyma of sometimes smaller, sometimes larger, cells with one to several nuclei and with extremely thin walls (fig. 15). It was often difficult to determine, whether adjacent nuclei were separated from one another by walls or not, as the walls were at places scarcely differentiated. In a number of anthers the pollen sacs were quite empty; only

a small amount of parenchyma had been formed, after which degeneration had set in, so that the decayed remains of the contents of the pollen sacs lay shrivelled up in the sac, whilst the wall of the anthers had continued its growth. In this abnormal case, which is a kind of unsuccessful apospory or somatisation of the microspores, the tapetal cells are only uninuclear, which likewise speaks in favour of the theory put forward above (p. 21—24) regarding the cytological conditions.

7. Growth of the pollen tube and the fertilization.

In order to determine experimentally, whether the pollen grain of the hops is able to germinate, I made a number of pollen cultures, not only of the two species of hops but also of other plants which could be useful for comparison. All the experiments were made in the laboratory at the room temperature.

On 1 per cent. agar solution neither the pollen grain of the common hops nor that of the Japanese hops showed any growth — nor when cane sugar was added to the culture liquid.

A decoction was then made of the styles of *H. lupulus*, but not even with this as nutriment basis was there any success in getting the pollen grains to germinate.

On 2 per cent. gelatine solution — both with and without addition of cane sugar — the pollen grains of *H. Japonicus* grew fairly quickly and in the course of 20 hours some of them developed pollen tubes of up to 10 times the diameter of the spore (fig. 16 *a*).

Thereafter experiments were made on *H. lupulus* with a 2 per cent. solution of mannito and the pollen of a perennial, red *Lathyrus* were used for comparison. Two series of experiments were made; in the one the sugar solution was made slightly acid by dipping in a crystal of tartaric acid, whilst in the other the solution was kept neutral. — After 20 hours only a few of the *Lathyrus* pollen grains showed any growth in the neutral solution and only with short tubes. In the acid solution, on the other hand, a number grew out with long, strongly twisted hyphae. The pollen of the hops showed no growth. After 45 hours there were still only a few of the *Lathyrus* pollen which showed any growth in the neutral solution, and the pollen grains of *H. lupulus* still lay unchanged at rest in both of the cultures.

As it thus seemed fairly difficult to induce any growth artificially in the pollen grains of the common hops, some of the female hops flowers were dusted over with pollen and their styles cut off and placed under the microscope after 4—5 days. A considerable number of growing pollen grains were then found on the styles, sending down pollen tubes of 6 μ thickness alongside the papillae of the stigma (fig. 16 *b*).

Further experiments were then made under conditions, which to a certain extent differed from the normal above-mentioned; a number of styles were cut from *H. lupulus*, sprinkled over with pollen by means of a brush and placed in a watch-glass with ordinary water. 20 hours later a number of pollen grains displayed very short hyphae 10—20 μ long (fig. 16 *c*). Yet it was only a small number; the growing pollen always lay near together and not in touch with the stigma papillae. After 44 hours some more seemed to have grown out; the hyphae were then up to 80 μ long, mostly thicker (12 μ) at the tip, elsewhere 6 μ wide. The diameter of the pollen grains was about 19—23 μ (fig. 16 *d*).

However much the pollen grains of the common hops showed themselves disinclined in these experiments to grow on quite artificial cultures, there was however no reason for doubting their power to normally grow down through the style and ovarial tissues of the female flowers. No doubt a suitable culture could be prepared in which growth of the pollen would take place. A chemical analysis of a cold water extract of hops styles in this connection would certainly give a helpful suggestion.

To determine to some extent, how long the pollen grains of the common hops retain their power to grow, inflorescences of the male plant were frequently collected and left to dry at the room temperature of the Laboratory. After 24 hours a fairly large quantity of pollen could be shaken out of the flowers and from this small portions were taken daily and used for dusting over flowers which previously had been enclosed in bags. These experiments proved, that the pollen of *H. lupulus*, even after being kept for 3 days, still possessed plenty of power to cause the fertilization of the female plant. — When we remember, that the pollen is distributed by the wind in the hops and that pollen, which is carried away by air currents, is probably under more favourable biological conditions than pollen which is kept

in a dry room in the laboratory, it is obvious, that natural pollination of the female plants may take place at a distance of hundreds of kilometers. We can thus understand, why it is that German and Austrian hops growers, even if they are most industrious in rooting out the male plants everywhere in their

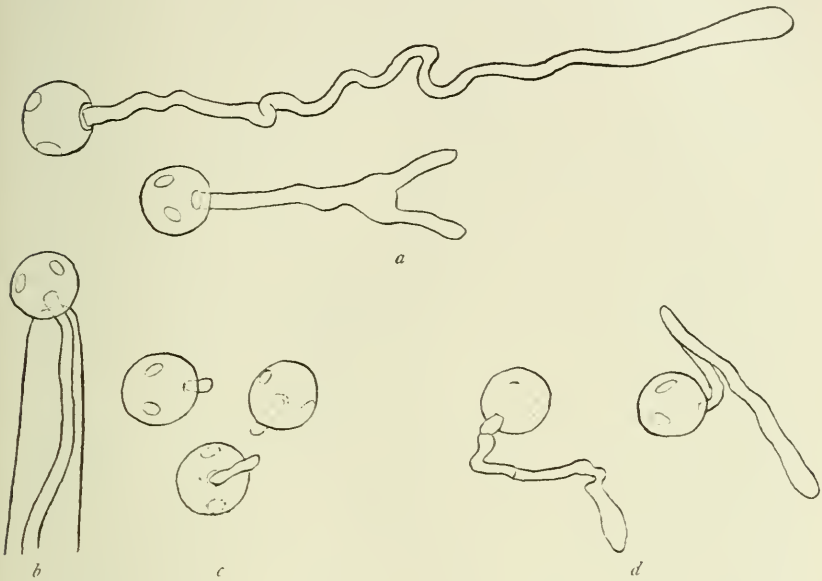


Fig. 16. *a. H. Jap.* Pollen growing on 2 per cent. gelatine solution. 20 hours after being sown. *b. H. lup.* Pollen growing on a stylar papilla. *c. H. lup.* Pollen growing in water containing *H. lup.* styles; 20 hours after being sown. *d.* The same 44 hours after being sown. (× c. 700).

districts, will nevertheless always find small quantities of fruit in their hops.

My investigations on the way along which the pollen tube penetrates down to fertilize the female sex-cell lead to the result, that Zinger's view quoted above (p. 3) is not altogether correct. Nawaschin's investigations on apogamous plants seem to have had a hypnotic influence on Zinger. As mentioned, he completely denies the presence of a micropyle in the hops, although this is normally present, as was shown earlier by Lermer and Holzner. I think it best to cite Zinger's statements (l. c. p. 242) regarding the penetration of the pollen tube in extenso.

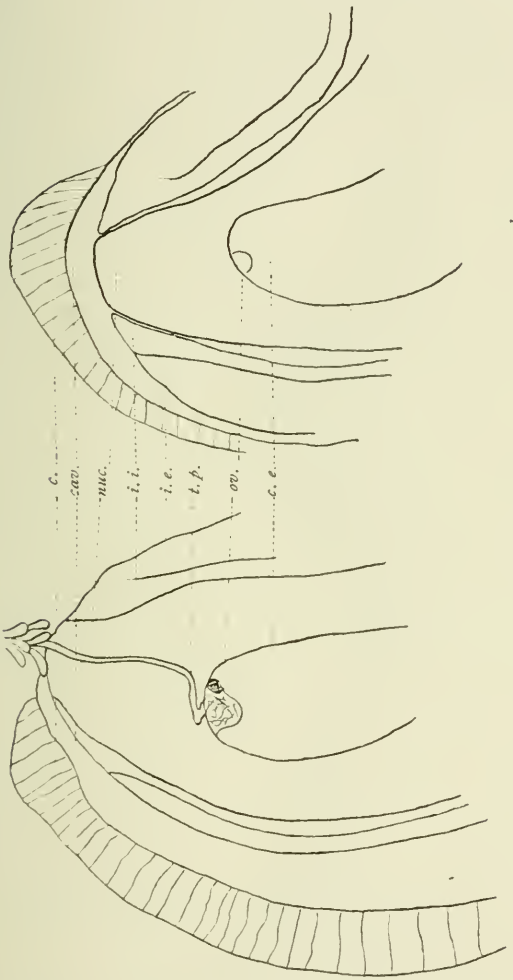
"Bei den *Humulus*arten drängt sich der Pollenschlauch im Griffel hinein und läuft in der oberen Wand des Fruchtknotens

genau in der für *Cannabis* angegebenen Weise. Bei *Humulus lupulus* ist er ebenso dünn, wie bei *Cannabis*. bildet jedoch die cellulösen Propfen viel häufiger. Während ich bei *Cannabis* stets nur einen Pollenschlauch im Fruchtknoten vorfand, sah ich in den meisten Fruchtknoten von *Humulus* je zwei oder drei Pollenschläuche. Nachdem der Pollenschlauch zur Samenknospe hinabgestiegen, dringt er bei dieser Pflanze in das Gewebe des äusseren Integuments ein; dabei zieht er sich meist in einer gewissen Entfernung von der Fruchtknotenhöhle hin, zuweilen auch knapp an deren Rand und tritt dann in das äussere Integument zwischen den rückwärts gerichteten Papillen ein. Darauf durchbohrt der Pollenschlauch den oberen Theil des äusseren Integuments, tritt an der Stelle, wo die Integumente mit einander verwachsen sind, in das innere Integument ein und erreicht durch dieses letztere die Nucellarspitze, ohne in die obliterirte Mikropyle zu gelangen. Auf diesem Wege nähert sich der Pollenschlauch der Oberfläche der Integumente, erscheint zuweilen selbst in der Fruchtknotenhöhle, um sich jedoch bald in die Tiefe des Gewebes wieder zu versenken. Sehr selten geht der Pollenschlauch so oberflächlich zwischen den Papillen der Integumente, dass er herauspräparirt werden kann. Ueber der Nucellarspitze bildet der Pollenschlauch von *Humulus* ebensolche sackartige Auswüchse und treibt ebenfalls einen dünneren Zweig in den Nucellus bis zum Embryosack hinein, wie wir es bei *Cannabis* beobachtet haben.

Bei *Humulus japonicus* ist der Pollenschlauch bedeutend dicker als bei *Humulus lupulus* und *Cannabis*. Meist steigt er sehr tief in die Gewebe des äusseren Integuments hinab, geht in das innere Integument gewöhnlich an der Stelle über, wo die Grenze zwischen den Integumenten noch deutlich zu unterscheiden ist, macht dort eine plötzliche Wendung nach oben und steigt durch das Gewebe des inneren Integuments bis zur Nucellarspitze der Samenknospe empor."

The possibility that Zinger's description, as Modilewsky (l. c.) briefly acknowledges, may be right for some cases, cannot naturally be denied, but it must be admitted, that the pollen tube in the flowers of both hops species pollinated and afterwards examined by me, only rarely penetrated so deep down into the outer integument, that the conditions could be said to agree with the description given above. With regard especially

to *Humulus Japonicus*, where Zinger finds that the pollen tube penetrates very deeply into the outer integument, I disagree with this investigator, for in most cases I find that the pollen tube goes in a straight direction from the base of the styles to the



17 a. 17 b.

Fig. 17. *a. H. Jap.* Apex of the ovule lying just opposite the conducting tissue; the pollen tube passing down without coming into contact with the integuments. *b. H. Jap.* The apex of the ovule has still not reached to beneath the conducting tissue; thus the pollen tube must grow through the apex of both integuments. *c.* innermost cell-layer of the ovarial wall; *cav.* ovarial cavity; *c. e.* embryo-sack; *i. e.* outer integument; *i. i.* inner integument; *nuc.* nucellus; *ov.* egg cell; *t. p.* pollen tube. (Apochr. 3 mm., comp. oc. 6; reduc. t. $^2/_3$).

tip of the nucellus without in any way touching the integuments. It is of some importance here, however, whether the ovule is very strongly bent or but slightly, since the tip of the nucellus in the most bent ovules reaches right opposite the point on the wall of the ovary where the conducting tissue ends, whereas in the less bent, younger ovules it is still some distance from there (cf. figs. 17 *a* and 17 *b*).

The growth of the pollen tube along a style papilla and into the style itself I find in agreement with Zinger's account and also the growth into the ovarial wall. The conducting tissue in *Humulus* is formed on the boundary of the two adjacent carpels, the extent of which can usually be readily distinguished even in pollinated ovaries. More or fewer pollen tubes lie close together in this tissue, the nuclei of which are remarkable by being far more basophile than the nuclei of the surrounding ovarial tissue. Sometimes on the boundary between the carpels there is actually formed a channel in which the pollen tube or tubes run. The conducting tissue is — and especially in *H. Japonicus* — closely compressed and sharply marked off, even deeper down in the ovary (figs. 4, 10). In *H. lupulus*, where the ovule is bent to a less extent than in *H. Japonicus*. the pollen tube extends beyond the boundary between the ovarial wall and the outer integument, which on the concave, posterior side of the ovule is developed into an extensive tissue (fig. 4). The part of this tissue towards the upper wall of the ovarial cavity often contains a continuation of the conducting tissue, in which the pollen tube continues its growth. The pollen tube still keeping close to the ovarial cavity penetrates further towards the apex of the ovule and passes over into the inner integument at the spot, where this has grown together with the outer, and pushes sideways into the micropyle. From this the pollen tube reaches the apex of the nucellus and finally the embryo-sack. As Zinger remarks, the pollen tube forms short branchings over the apex of the nucellus before a single branch is sent down towards the embryo-sack. — There is nothing in *H. lupulus*. it appears, which indicates true aporogamy. The pollen tube takes the shortest possible way to the embryo-sack and — though in a somewhat peculiar manner — even passes through a micropyle which is usually distinctly developed. That the ovarial cavity is avoided as a rule, is connected I believe with phylogenetic conditions, which may be considered more closely. — If we take the conditions in *H. Japonicus* we find, that the pollen tube has a much easier approach to the nucellus, the apex of which is naked here, uncovered by the integuments, and owing to the great curvature of the ovule, lying just opposite the conducting tissue (figs 10, 17 *a*). In certain cases, for example when the ovule is young and thus less curved (figs. 17 *b*. 18), the apex of the nucellus is

not yet placed right opposite the conducting tissue in the wall of the ovary. Nevertheless the flower is ripe for pollination and thus the pollen tube, to reach the nucellus, must pass through the uppermost part of the integuments. I must conclude from

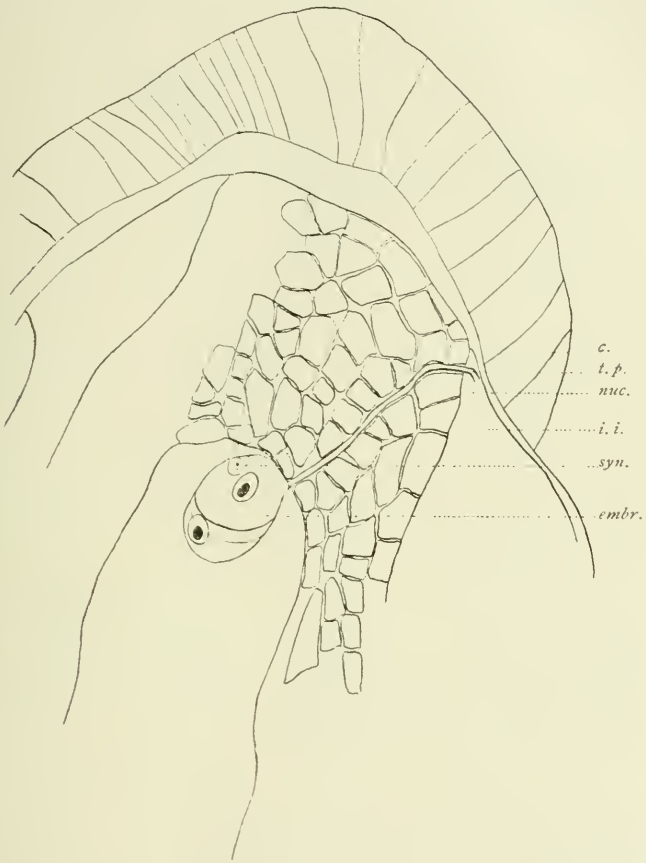


Fig. 18. *H. Jap.* Apex of the ovule shortly after fertilization. The pollen tube has grown through the apex of the inner integument. *c.* innermost cell-layer of the ovarial wall; *embr.* embryo; *i.i.* inner integument; *nuc.* nucellus; *syn.* synergid; *t.p.* pollen tube.

this, that in *Humulus* the age of the ovule has just a great influence on the penetration of the pollen tube, and that it is due to the fact, that the ovaries of both species become ripe for fertilization before the ovule is so bent that the apex of the nucellus lies opposite the conducting tissue in the ovarial wall, that the manner of penetration through the integuments has been changed. We must suppose, therefore, that the chemotactically

active substances which attract the pollen tube, pass through the integuments in agreement with the "too early" maturation of the ovule. To correspond with this the anatomical structure of the outer integument has changed in such a manner, that cells of quite a similar appearance to those occurring in the original conducting tissues of the ovarial wall, can be observed in the strongly developed, posterior side of the outer integument. How far the growing together of the integuments at the apex — and especially on the posterior side of the ovule — may also be considered as an adaptation to facilitate the growth of the pollen tube, may be left unsettled. That the pollen tube can quite well grow through the narrow ovarial cavity, when pollination takes place so late, that the apex of the nucellus has become bent right up under the conducting tissue, I have observed in *H. Japonicus* (fig. 19).

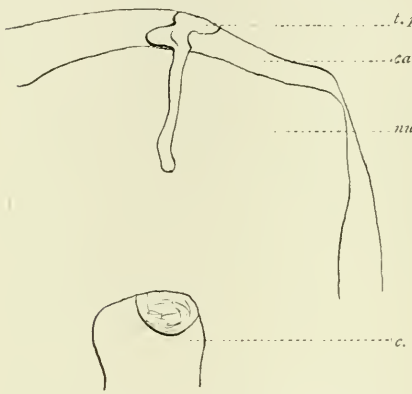


Fig. 19. *H. Jap.* The pollen tube grows through the narrow ovarial cavity just over the apex of the nucellus. *cav.* ovarial cavity; *c. e.* embryo-sack; *nuc.* nucellus; *t. p.* pollen tube.

When fertilization has occurred — I have only succeeded in observing the fertilization of the egg-cell — the embryo begins its divisions transversally and the nucleus of the endosperm likewise begins dividing. Modilewsky (l. c.), as already mentioned in section 2, finds that two kinds of endosperm nuclei occur in the embryo-sack (in *H. Japonicus*). "Die meisten, welche im oberen und mittleren Teile des Embryosacks liegen, sind oval, ziemlich gross und besitzen stets einen grossen Nucleolus. Im antipodalen Teile, dicht im Protoplasma eingebettet, befinden sich etwas grössere Kerne mit viel dünnerer Membran. In jedem Kerne sind zwei bis drei Nucleolen vorhanden, aber ziemlich klein und schwach gefärbt. Das Kerngerüst dagegen ist intensiv gefärbt und deutlich differenziert, während bei gewöhnlichen Endospermkernen es fast unsichtbar ist. Gewiss haben diese Kerne hier dieselbe Funktion bei der Ernährung wie bei den Urticaceen" (p. 459). In the Urticaceae

Modilewsky finds small haustorial organs developed, but in most cases reduced to a mass of "haustorial nuclei" in the basal part of the embryo-sack. — I can only say, that neither in *H. lupulus* nor in *H. Japonicus* do I find two kinds of nuclei like those mentioned by this author. Most of the endosperm nuclei

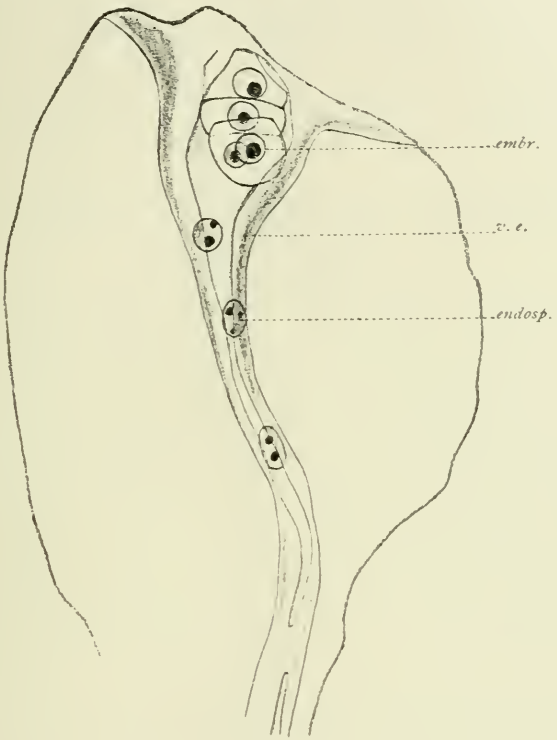


Fig. 20. *H. Jap.* Part of the embryo-sack. The outermost cell in the young 3-celled embryo has divided by a longitudinal wall. Only 3 endosperm nuclei visible in the embryo-sack. *embr.* embryo; *endosp.* endosperm nuclei; *v. e.* shrivelled embryo-sack wall. Hom. imm. 2 mm., comp. oc. 4; reduc. t. $^2/_3$.

have 2 or 3 nucleoli, also in the upper part of the embryo-sack. The antipodes degenerate without further divisions. Rather few endosperm nuclei are commonly formed. The embryo shortly afterwards consists of a row of cells, 3—4 cells long (fig. 20), in which longitudinal walls then arise. For the further development of the embryo reference may be made to Lermer and Holzner's description (l. c.) and it need only be remarked here, that the embryo at the time the fruit is ripe is greatly rolled up;

the one cotyledon being rolled over the germ and the other cotyledon, thus enclosing the whole body of the embryo.

In *H. Japonicus* the period from pollination till fertilization lasts normally 70 hours (in our hothouse) and the fruit is often ripe in 14 days. In *H. lupulus*. on the other hand, about twice as long is required before fertilization occurs and the fruit takes almost always a month to become ripe. Naturally, however, the weather plays a no small part here.

We have now still to consider some experiments which were made to produce a hybrid between the two species of hops.

8. Experiments to produce a hybrid between *H. lupulus* and *H. Japonicus*.

During the artificial pollination experiments which have been carried on for some years in our experimental garden and hothouse, I tried repeatedly to produce hybrids between the two hops species. Although it had to be recognised beforehand, that this was hopeless owing to the great biological differences between the species, I persisted, for in addition to the possibility of really producing the hybrid there was the hope of being able to solve problems of great general interest. *H. lupulus* is a perennial, *H. Japonicus* an annual plant and in addition there are many other great, inner and outer differences between the two species. To ascertain what a hybrid between such plants would be like, seemed undeniably worth the experiment. It may at once be said, that the attempts were only successful to a very limited extent, but as a certain amount of interest lies in the results, they may be discussed here.

Pollination of previously enclosed female inflorescences was made on a fairly large scale, partly with *H. lupulus* as mother and *H. Japonicus* as father, partly the converse. It was soon evident, that the pollen of the common hops had exercised no influence on the female flowers of *H. Japonicus:* these remained unchanged and it may perhaps be taken, that the pollen had not even grown out under these, somewhat unnatural conditions. In the pollen growth experiments already described, it will be remembered, the pollen of *H. lupulus* made more special claims on the environment than the Japanese hops. On the other hand, when the pollen of the latter was transferred to the ripe styles

of *H. lupulus*. these in the course of some days became brownish. then shrivelled up and fell off, after which the ovary became swollen. After 20 days the ovary had assumed quite a considerable size, almost of the normal dimensions of the ripe hops nuts, and after another 2 weeks they had the appearance of being quite like the normal nuts. At the same time their hops had grown out in the usual manner, whilst hops on the same plant, which had not been pollinated, had less developed leaves and axes, as the growth had stopped early. A closer macroscopic analysis of these fruits showed, however, that their interior was empty. With the lens it could be seen, that the embryo-sack had developed, but no embryo was to be seen. Then well-fixed material of the artificially pollinated ovaries was frequently taken in the course of the experiments in order to determine if possible what had happened, and the result of the investigation was as follows.

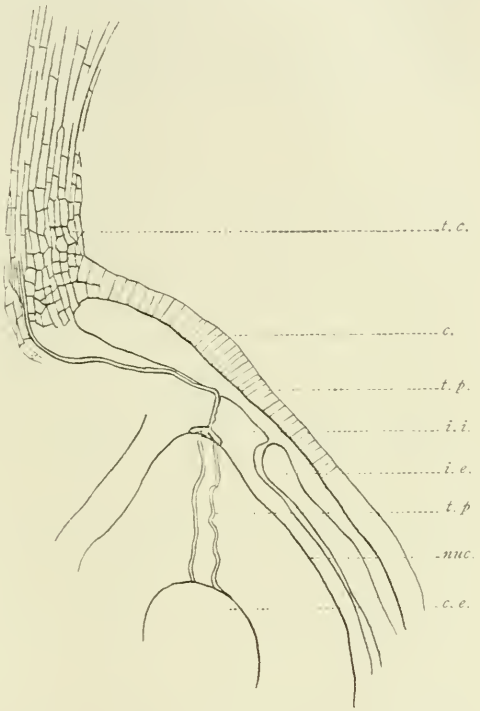
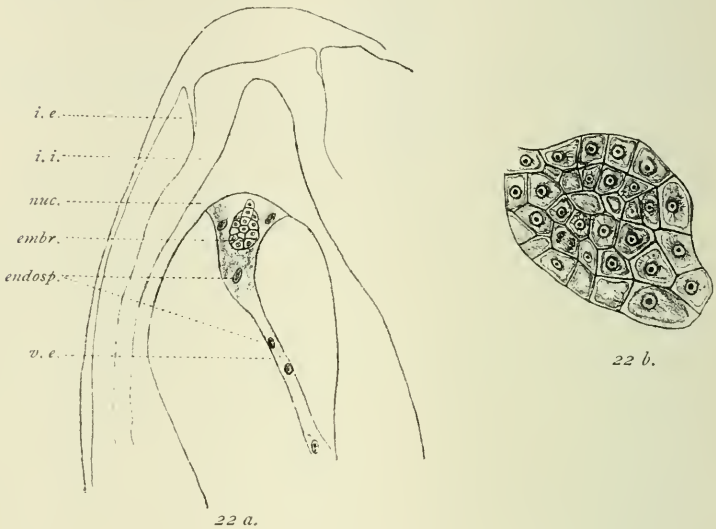


Fig. 21. *H. lup.* fertilized with the pollen of *H. Jap.* Almost median longitudinal section of the apex of the ovule. The pollen tube has penetrated through the integuments which have grown together at the top and has entered the micropyle. Two, probably different pollen tubes reach down to the embryo-sack. *c.* innermost cell-layer of the ovarial wall; *c. e.* embryo-sack; *i. e.* outer integument; *i. i.* inner integument; *t. c.* conducting tissue; *t. p.* pollen tube. (Apochr. 8 mm., comp. oc. 4; reduc. t. 1/2).

The pollen grain of the Japanese hops had grown out and the pollen tube had grown down through the styles and the conducting tissue, then further through the integuments and had reached the micropyle and from there the nucellus and the egg-cell (fig. 21), in fact, had acted in exactly the same way as the true pollen of *H. lupulus*. As a rule ferti-

lization had taken place in the embryo-sack and a small embryo had developed (fig. 22); further, an endosperm of up to 2—3 hundred cells was present as a rule. The embryo however, had never attained to any greater development. In the largest hybrid embryo about 200 cells could be counted. Sometimes a well-developed endosperm was found, without any developed embryo being formed, and the fertilized egg-cell then lay shrivelled at the micropylar end of the embryo-sack. In other cases only



22 a.

22 b.

Fig. 22. *a. H. lup.*, fertilized by pollen of *H. Jap.* Nearly median longitudinal section of apex of the ovule. A small bastard embryo and endosperm formed. *b. H. lup.* × *H. Jap.*, longitudinal section of bastard embryo. *embr.* embryo; *endosp.* endosperm; *i. e.* outer integument; *i. i.* inner integument; *nuc.* nucellus; *v. e.* shrivelled wall of embryo-sack. (*a.* Apochr. 8 mm., comp. oc. 4; reduc. t. $^2/_3$. *b.* Hom. imm. 2 mm., comp. oc. 2; reduc. t. $^2/_3$).

one endosperm nucleus could be found, lying in the greatly shrunken nutriment contents of the embryo-sack. — Thus the hybrid *H. lupulus* × *H. Japonicus* can actually be formed but obviously lacks the possibilities for existing as a fully developed plant. Apparently, material disagreements appear very early already between the elements of the two species in the hybrid, as one would expect. In no cases have I been able to determine the number of chromosomes in the hybrid, but there can hardly be any doubt, that there are 10 + 8 = 18 chromo-

somes. The number of chromosomes in the hybrid endosperm (fig. 55) could not be determined either.

It seems to me therefore in so far possible, that the earlier mentioned statement (p. 2), which emanates from a practical man, that the pollen of *Urtica* may produce fruits in the cultivated hops, may be correct. *Urtica* is certainly more remote from *H. lupulus*, but the statement in itself is not to be rejected.

9. Summary.

The result of the investigations may summed up as follows.

Zinger's description of the formation of the embryo-sack and the growing together of the integuments in *Humulus* is correct, but the author is wrong in his statement, that the ovule lacks a micropyle, the presence of which has already been determined by Lermer and Holzner.

The microspores are developed and ripen in basipetal order in correspondence with the dehiscence of the pollen sacs by means of apical pores.

It is not easy to get the pollen grains of *H. lupulus* to grow on an artificial substratum, but the pollen grains of *H. Japonicus* grow willingly on a gelatine solution.

The pollen grains of *H. lupulus* retained their power of growth for 3 days on being kept in a dry room of the laboratory.

The tapetum in *Humulus* offers a good example of multinuclear cells and vegetative caryogamy, the original divalent nuclei dividing repeatedly, after which the products of division constantly fuse together. This gives rise to plurivalent, synkaryon-like nuclear complexes.

Rosenberg's and Bonnet's theory, that the tapetum originates phylogenetically from the archespore and consists of sterile archespore cells, cannot be accepted, and for this reason it cannot be admitted either, that atavistic tendencies give the explanation of the mutinuclear condition of the tapetal cells. It is suggested, that the peculiar nuclear condition of the tapetum has a physiological explanation and the same applies to the endosperm's wealth of chromosomes. Both tissues are to a very great extent connected with the nutrition, which must be taken to be reflected in the abundance of chromatin.

The growth of the pollen tube in *Humulus* does not suggest

true aporogamy, as Zinger maintains. Zinger's description of the deep penetration of the pollen tube into the integuments is somewhat exaggerated, especially in the case of *H. Japonicus*. The more or less advanced age of the ovule at the moment of pollination affects the route by which the pollen tube penetrates to the nucellus, the ovule in younger flowers being less curved than in older, yet nevertheless ripe for fertilization. In younger flowers the pollen tube must pass a longer way through the integuments. In certain cases — in older flowers of *H. Japonicus* — the pollen tube passes directly by the conducting tissue to the nucellus without touching the integuments.

The number of chromosomes in the somatic cells is 20 and 16 in *H. lupulus* and *H. Japonicus* respectively; in the x-generation respectively 10 and 8. In the tetrad formation two longitudinal divisions of the chromosomes are observed, but no transverse division.

In abnormal, monoecious hops plants of *H. lupulus* it has been found, that the reduction division proceeds in the pollen mother-cells; but in the observed cases the gonotokonts then became starved, the tapetum being already degenerated.

In an abnormal, gynomorphous male plant of *H. lupulus* no gonotokonts developed at all, but the microsporangia were filled with sterile tissue.

It proved possible to produce the hybrid *H. lupulus* × *H. Japonicus*, but only as imperfectly formed embryo. The pollen tube of *H. Japonicus* grew down into the ovary of *H. lupulus* in the same way as the pollen tube of *H. lupulus* itself, and the bastard embryo and bastard endosperm or only one of them developed.

Humulus did not develop seed without fertilization in the experiments which were made.

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EXPLANATION OF FIGURES.

All the figures in the text and on the Plates have been drawn by means of Abbe's apparatus. The figures given on the Plates were all drawn with the same magnifications, Zeiss homog. immers. 2 mm., comp. oc. 18. On reproduction the figures were reduced to $^5/_6$.

Fig. 23. *H. lup.* Cell from ovarial wall. 20 chromosomes in metaphase.
24. *H. Jap.* Archespore mother-cell. 16 chromosomes in anaphase.
25. *H. Jap.* Tapetal cell with dividing nucleus. Anaphase.
26. *H. Jap.* Tapetal cell in two sections. Peculiar diakinesis-like prophase. Apparently 15 whole pairs of chromosomes present, one of which has been carried by the microtome knife outside the nuclear membrane, and 5 single chromosomes. Of these 5 there are 2 and 2 in pairs, but the half parts in section *a* have been moved a little by the knife; the fifth must be supposed to be a portion of a divided chromosome. Thus in all there are apparently 17 pairs of chromosomes, but the excess has probably arisen from one of the 16 pairs being cut across.
27. *H. Jap.* Prophase in two already hyperchromatic tapetal cell nuclei.
28. *H. Jap.* Hyperchromatic tapetal cell.
29. *H. Jap.* Tapetal cell with resting synkaryon. Chromosomes dissolving.
30—31. *H. lup.* Pollen mother-cells with nuclei in synapsis.
32. *H. Jap.* Pollen mother-cell. Postsynaptial stage with U-shaped chromatin slings (pair of chromosomes).
33. *H. lup.* Pollen mother-cell. The chromosome pairs shortened. Haematoxylin preparation, very long differentiation in iron-alum.
34. *H. Jap.* Pollen mother-cells. The partly twisted pairs of chromosomes are becoming shorter.
35. *H. Jap.* Pollen mother-cell. Ring-shaped pairs of chromosomes.
36. *H. Jap.* Pollen mother-cell. 8 U- and Y-shaped chromosome pairs.
37. *H. Jap.* Pollen mother-cell. Chromosomes in each pair separate. Diakinesis.
38. *H. lup.* Pollen mother-cell. Reduction division. Late metaphase.
39. *H. lup.* Pollen mother-cell. Same stage. Detailed picture of the chromosomes.
40. *H. lup.* Pollen mother-cell. Reduction division. Late anaphase.
41. *H. lup.* Pollen mother-cell. Anaphase of the reduction division.
42—43. *H. lup.* Pollen mother-cell. Anaphase viewed from the one pole.
44. *H. Jap.* Pollen mother-cell. The reduction division. Early anaphase. Only the one nuclear plate apparent.
45. *H. lup.* Pollen mother-cell. Telophase of the reduction division.
46. *H. Jap.* Pollen mother-cell. Prophase of the homoeotypical division. Diakinesis.
47. *H. Jap.* Pollen mother-cell. Metaphase of the homoeotypical division.
48. *H. Jap.* Pollen mother-cell. Anaphase of the homoeotypical division.
49. *H. Jap.* Pollen mother-cell. Telophase of the homoeotypical division.
50. *H. Jap.* Young pollen tetrad.
51. *H. Jap.* Almost ripe pollen tetrad.
52. *H. lup.* Pollen grain. The first generative division in the pollen grain has taken place.
53—54. *H. lup.* Pollen mother-cell of monoecious plant. Diakinesis.

Printed 6. August 1914.

POSTSCRIPT.

After the present work was printed, the University Botanical Library opened again some few days ago after the summer vacancy, and by the kindness of Professor Eug. Warming I learned that in the meantime an interesting treatise had been published by Julien Tournois: »Études sur la sexualité du Houblon« (Annales des Sc. Nat., T. XIX, 1914, p. 49—191) which, as may be seen from the title deals with the same question that I have investigated. In the named work I find that the author already in 1911 has made a provisional short communication, that he has succeeded in producing germs in *Humulus lupulus* by dusting the female flowers with pollen of *Cannabis sativa*. This paper, »Formation d'embryons chez le Houblon par l'action du pollen du Chanvre« (C. R. Ac. Sc. Paris, T. CLIII, p. 1160—1162) unfortunately I had not noticed, as I have had no occasion to read this periodical regularly, but know only the contents from »Botanisches Centralblatt« — and in this journal the work has not been reviewed.

Julien Tournois' new explicit work deals mainly with the anomalies in the distribution of the sex and the outside conditions' influence on it, as also the influence of the fruitformation on the formation of hops.

Allthough Tournois' and my results agree mostly with each other where the researches deal with the same subject, there is a little difference in some particulars.

Tournois finds, f. i, 10 chromosomes in the gametophyte both in *H. lupulus* and *H. Japonicus*, while I, as it among others will be seen from my tables, in the latter find only 8 — and in agreement herewith 16 in the sporophyte. Tournois, however, does not seem to have investigated thoroughly this particular, and I have no doubt that a closer examination will show the author that my statement is the correct one.

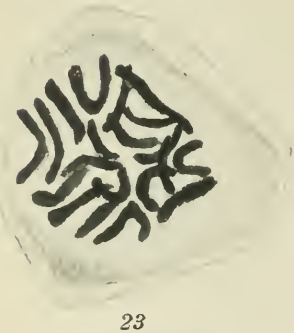
Tournois finds the anthers empty in monoecious hops plants (»Early Prolific«), and he thinks on the whole that it is doubtful if true hermaphroditism is present at all — and that the separation of the two sexes biologically is completely realized. From the statement (p. 27—28) in my paper it will be seen that my opinion is different and it may be added that this summer I have been able to make pollinations with apparently quite normal pollen from a monoecious hops.

No more I can admit (see p. 40) the statement of Tournois (p. 159) that the endosperm is fully disappeared in *H. lupulus* after a cross-pollination with *H. Japonicus*.

The statement of Tournois, that the formation of an embryo in the ovaries of *H. lupulus* after pollination with *Cannabis sativa*-pollen, most frequently should be parthenogenetic (p. 162) deserves attention. A more thorough investigation must, however, be required before this question can be entirely settled.

Copenhagen, 31st August 1914.

ÖJVIND WINGE.



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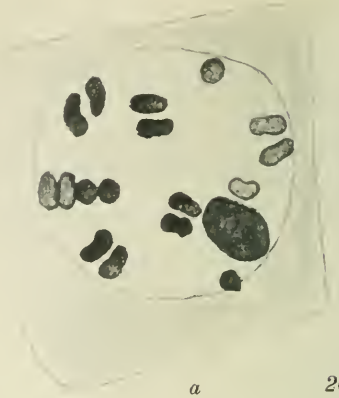

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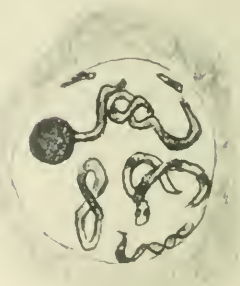

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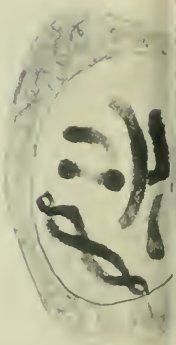

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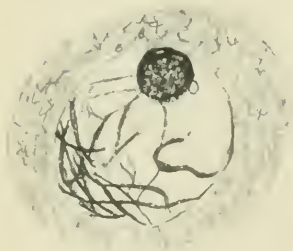

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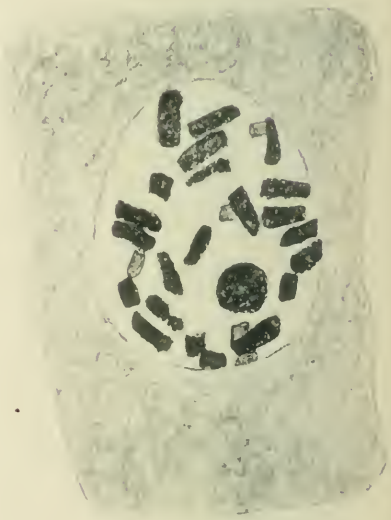

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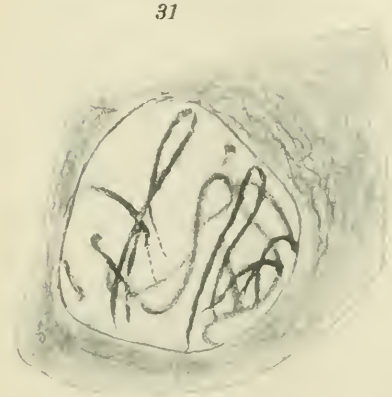

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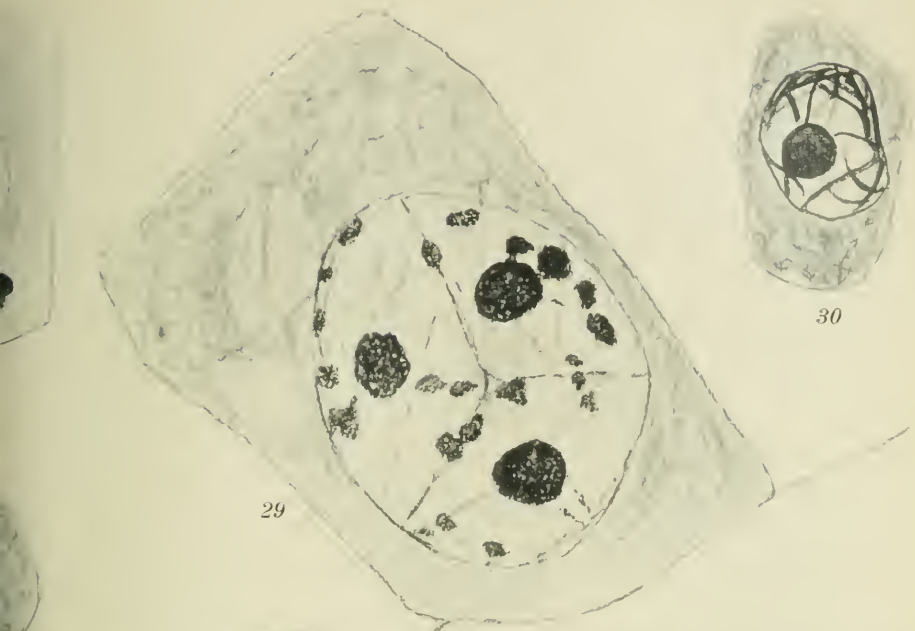

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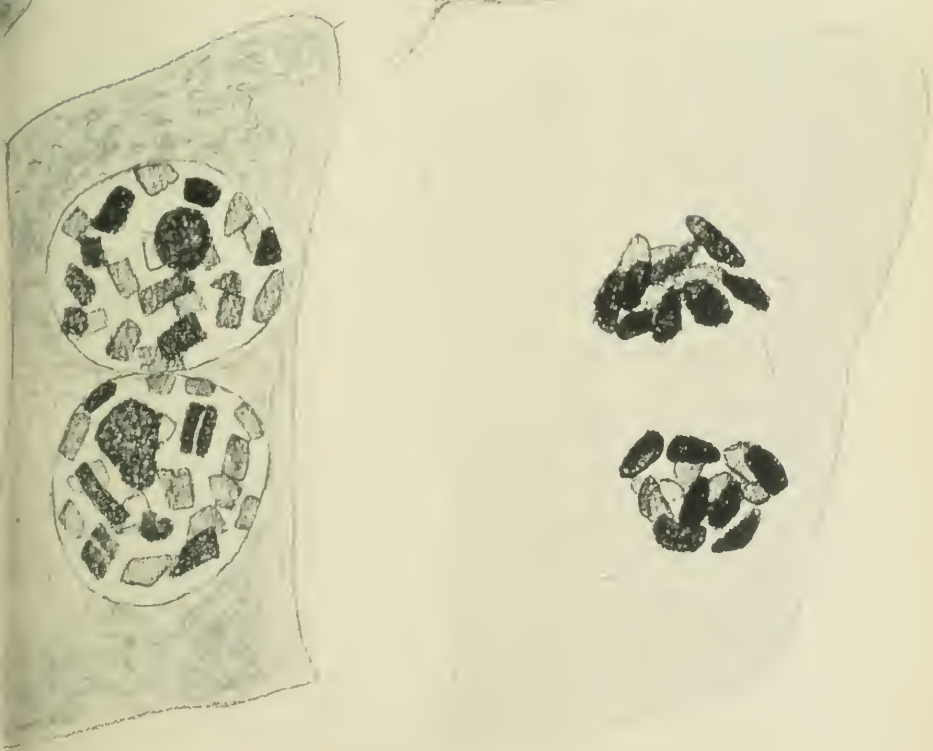

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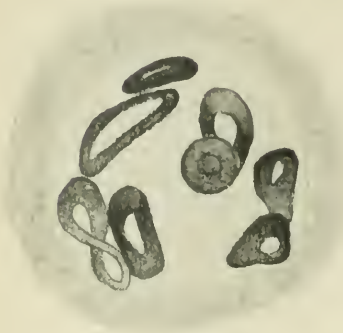

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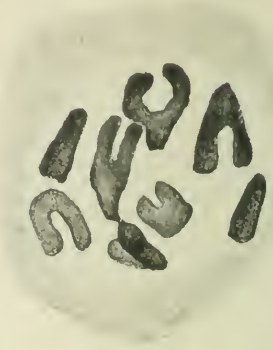

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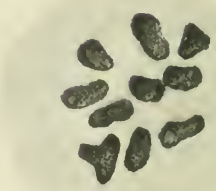

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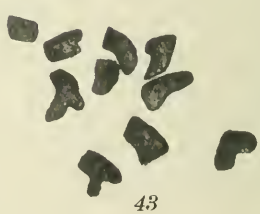

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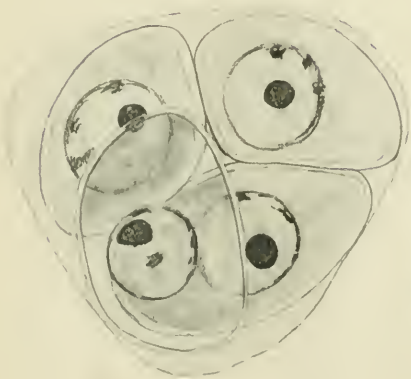

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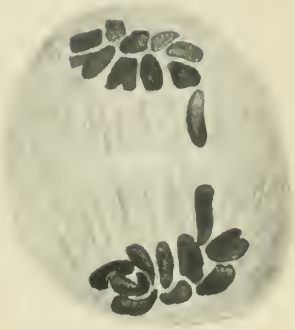

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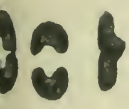

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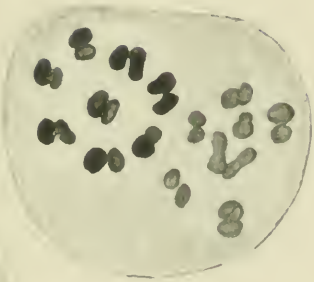

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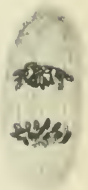

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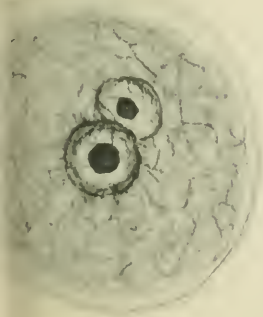

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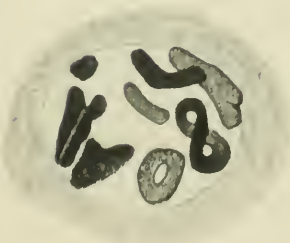

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ON THE PROTEID SUBSTANCES OF BARLEY, IN THE GRAIN ITSELF AND DURING THE BREWING PROCESSES.

BY

H. SCHJERNING.

AFTER my researches concerning this subject were brought to a termination, being published in the Comptes-rendus of the Carlsberg Laboratory of the years 1906—1910—1913, I have been called upon by the Board of Directors of the Laboratory to give a summary view of the whole work. I am much pleased to comply with this request because my more recent investigations have in various ways thrown some light upon the preceding sections, and also because I am furnished an opportunity of giving a complete description of the method by which the quantities of the different groups of proteïd substances or proteïd individuals were estimated. The fact is that this method forms the basis of my work, and was never yet published in connection with the main work. A joint description of the analytical method employed is all the more desirable because the latter has been from time to time modified and improved in certain points, so that whenever information is wanted on the precipitants and methods of precipitation I shall have to refer to more than one of my publications.

In each section of the summary here given I will refer the reader to my original memoirs, where the bibliography of the subject is mentioned and a full description given of my experiments and the results obtained by these. The references will in the text be parenthesised, stating volume and page of the Comptes-rendus, for example — (**8**, 207).

The analytical Method of Determination of the different Groups of Proteïd Substances.[1])

As a starting-point and basis of the method adopted serves a fact the correctness of which is borne out by numerous experiments, namely that any proteïd groups or proteïd individual, of whatever origin it may be, can be precipitated by one or several definite precipitants, the inter-relations of which have been established by experiments.

The proteïd solution to be examined should preferably have a nitrogen concentration of 7—10 mgr. of nitrogen to 10 cc. of proteïd solution[2]). It will then be advisable to take 20—25 cc. of proteïd solution for each determination or precipitation. A more concentrated proteïd solution may conveniently be diluted with distilled water till the above concentration is reached, as it is not recommendable to undertake the analytical precipitations in solutions of a nitrogen concentration exceeding to an appreciable extent the above limit. In the case of a very dilute proteïd solution a correspondingly larger volume may be used for each precipitation.

It must further be watched that the proteïd solution to be examined shows a slightly acid reaction. A suitable acidity is reached when 25 cc. of proteïd solution, after 2 cc. of a (1 %) solution of phenolphtaleïne has been added, shows a distinctly red coloration on the addition of 1—4 cc. of $^1/_{10}$ normal solution of sodium hydroxide. If the proteïd solution has an alcaline reaction, dilute phosphoric acid is added, and if it contains too large a proportion of free acid, this must be partially neutralised by means of a solution of barium hydroxide, — in either case to such an extent that the acidity will be comprised between the above-mentioned limits.

If the proteid solution contains larger quantities of soluble salts, particularly of light metals, the precipitations with sublimate,

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[1]) With regard to the preliminary and fundamental research-work, I may refer to the Zeitschr. f. anal. Ch. **33**, 263 (1894); **34**, 135 (1895); **35**, 285 (1896); **36**, 643 (1897); **37**, 73 and 413 (1898); **39**, 545 (1900), and to the Compt. rend. des travaux du Laborat. de Carlsb. **8**, 207 and 210 (1910).

[2]) Accordingly, 10 cc. of proteïd solution treated by Kjeldahl's method will require 5—7 cc. of $^1/_{10}$ normal acid to neutralise the amount of ammonia formed.

ferric acetate and uranyl acetate may become partially reciprocal and, consequently, quantitatively precarious.

The proteïd precipitates formed are always collected on a filter of 11 cm. which has been previously washed with hydrofluoric acid[1]) (Scheicher & Schüll No. 589, with white band), where they are washed, generally twice, with the special liquid. Subsequently, after the filter has been conveniently folded, a considerable part of the washing liquid left in the very voluminous precipitates can be removed by gently pressing with the fingers. The filter and precipitate are then brought back to the flask in which the precipitation was effected, where they receive an addition of concentrated sulphuric acid and are treated completely by the well-known method of Kjeldahl. By a large number of estimates such a filter was found to contain, on an average, a nitrogen quantity corresponding with 0.2 cc. of $^1/_{10}$ normal acid, a figure which has accordingly to be introduced as correction for each proteïd precipitation.

The precipitations are best effected in Jena flasks of a suitable size (Kjeldahl's flasks), so as to admit of being washed with the liquid in question and to enable the washed proteïd precipitate to be further treated as mentioned above. In ferric acetate precipitations it is advisable to use flasks of 400—500 cc., in the other precipitations such of 100 cc.

The reagents required for the quantitative determination of the different groups of proteïd substances or proteïd individuals existing together in a solution are the following: —

(1) A stannous-chloride solution prepared by dissolving 50 gr. of rasped tin in a copious quantity of conc. hydrochloric acid in a tared flask. After the mixture has received an addition of some drops of a platinum chloride solution, the flask is covered with a watch-glass and left to stand at the ordinary temperature of the room till by far the greatest part of the platinum has been

[1]) The filter must first be thoroughly moistened with distilled water and placed in the funnel in such a manner as to make the upper edge of the filter join tightly on to the wall of the funnel. In the case of the precipitation with magnesium sulphate, however, the filter must be soaked with a saturated solution of magnesium sulphate (Reagent No. XI). It is further to be observed that in all the precipitations, excepting that with ferric acetate, it is advisable to pour back into the precipitation flask the 25 cc. of filtrate which have first run off. To obtain a perfectly clear filtrate, it is now and then necessary to pass the liquid several times through the same filter.

precipitated out. Then the mixture is slightly warmed until the tin has been entirely dissolved. Subsequently the solution is evaporated[1]) till the weight has been reduced to 130 gr. If the evaporation has exceeded this limit, a small portion of conc. hydrochloric acid may be added and the evaporation thereafter continued till the weight of 130 gr. is attained. After an addition of about 300 cc. of boiling distilled water, the solution is passed as quickly as possible through a small folded filter into a measuring flask, where it is made up to 1000 cc. with boiling distilled water. It is then, after being shaken, poured into some small white vials, which must previously have been boiled out with dilute hydrochloric acid, washed with distilled water and warmed in a hot-air oven. As soon as the vials have been entirely filled with the warm stannous-chloride solution, they are closed with India-rubber stoppers, which must have been washed in the same manner as the vials. It will be the best plan to choose such a size that the contents correspond with the amount wanted on the very day on which the vial is to be opened. The vials should be preserved in a horizontal position, and for the purpose of obviating the access of oxygen it will be well, immediately after filling them, to coat the neck and stopper over with paraffine. The stannous-chloride solution must be quite clear and not contain any basic tin combination. It should contain, per 100 cc., about 8 gr. of $SnCl_2$ and 1.28 gr. of free hydrochloric acid (HCl). 2 cc. of solution to which some drops of phenolphtaleïne solution have been added, must require about 24 cc. of an $^1/_{10}$ normal solution of sodium hydroxide to give a distinct red colour.

A stannous-chloride solution which has been in contact with the air, even though only for a few hours, should not be used. The same remark applies to a solution containing platinum that has not been thrown down, and which manifests itself by imparting a yellow or yellowish tint to the liquid. As for the length of time through which the tin solution can keep its power of precipitation, it depends upon how far the access of oxygen has been prevented during the preparation and preservation of the solution. As long as the latter is clear, it is fit for use. A slight secretion of stannous sulphide (black), due to the sulphur con-

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[1]) During the evaporation the flask must remain covered with the watch-glass in order to prevent the access of the oxygen of the air.

tained in the India-rubber stoppers, does not matter. — Reagent No. I.

(2). A neutral or slightly acid, clear solution of calcium chloride, containing some 10 % of $CaCl_2$. — Reagent No. II.

(3). A corresponding solution of calcium chloride, containing only about 1 % of $CaCl^2$. — Reagent No. III.

(4). Pure cristallised mercuric chloride. As this reagent must not contain either basic salt or free hydrochloric acid, it will be recommendable to use the pure preparation of Kahlbaum »Zur Analyse«. A clear 5 per cent solution in distilled water should be prepared from this. — Reagent No. IV.

(5). Pure and dry lamellated ferric acetate, 0.8 gr. of which must give a clear or almost clear solution with 50 cc. of distilled water at 50^0—100^0. — Reagent No. V.

(6). An aqueous solution of secondary sodium phosphate, containing 0.4 % of Na_2HPO_4, 12 H_2O. — Reagent No. VI.

(7). Dilute acetic acid, containing 15 cc. of 45 per cent acetic acid per litre. — Reagent No. VII.

(8). Pure uranyle acetate. The salt should be as free from sodium as possible; it must not contain nitrogen in any form whatever, and neither basic nor acid salt in considerable quantity. On the other hand, it need not be anhydrous. 6 gr. of the salt, if dissolved in 100 cc. of distilled water at 50^0, should give a clear liquid. Kahlbaum's preparation »Uranylacetat, natriumfreies« appears to be the most suitable. If the salt cannot be procured in another way, it may be prepared by the method suggested by C. Dittrich[1]).

A clear 6 per cent solution in distilled water should be prepared. — Reagent No. VIII.

(9). Pure cristallised magnesium sulphate — $MgSO_4$, 7 H_2O. Kahlbaum's preparation »Zur Analyse« answers the purpose. — Reagent No. IX.

(10). Dilute sulphuric acid, prepared by mixing equal quantities of conc. sulphuric acid and distilled water. — Reagent No. X.

The doses to be added in the salting-out precipitations with magnesium sulphate are: —

[1]) Die Uranylsalze, pages 4—7. Inaugur. Diss. Leipzig 1899.

To 10 cc. of proteïd solution:		4 drops of dilute sulphuric acid.	
- 20	—	8	—
- 25	—	10	—
- 30	—	12	—

and so forth.

(11). A saturated solution of magnesium sulphate, containing about 0.5 % of free sulphuric acid.

2 kgr. of cristallised magnesium sulphate — $MgSO_4$, $7H_2O$ Ph. D. — are dissolved at about 50° in the necessary quantity of distilled water. After being cleared by filtering, the solution is made up to 3 l. with distilled water. The saturated solution then receives an addition of 15 gr. of conc. sulphuric acid, and is stirred up well. — Reagent No. XI.

The precipitations are effected as follows: —

For a proteïd precipitation to be satisfactory, it is necessary that the liquid, when filtered from the precipitate, should be quite clear. In the case of stannous-chloride, however, this demand cannot be fulfilled entirely, because the filtrate, by coming in contact with the oxygen of the air, will sooner or later become turbid in consequence of the formation of basic stannic salts. In such a case it can, of course, only be demanded that the liquid must be perfectly bright immediately after being filtered. In regard to the ferric acetate precipitation care must be taken to have all the iron thrown down, so that the filtrate does not contain any ferric salt.

Stannous chloride precipitation. To 25 cc. of proteïd solution of the above-mentioned concentration and acidity are added 5—10 cc. of calcium chloride solution (Reagent No. II) and 20—25 cc. of stannous chloride solution (Reagent No. I). The mixture is shaken well and left to stand till the next day in a dark cupboard at the ordinary temperature of the laboratory. The sediment is then collected on a filter, and the flask and sediment are washed twice with a dilute solution of calcium chloride (Reagent No. III) to which have been added a few drops of the stannous chloride solution.

Mercuric chloride precipitation. To 25 cc. of proteïd solution is added about 50 cc. of mercuric chloride solution

(Reagent No. IV)[1]). The mixture is shaken well and left to stand all night in a dark place at the temperature of the laboratory. The sediment is collected in the normal manner and washed, together with the flask, with the precipitant itself (Reagent No. IV)[1]).

Ferric acetate precipitation. Into the precipitation flask are weighed out about 0.8 gr. of ferric acetate (Reagent No. V), to be dissolved in some 40 cc. of dilute acetic acid (Reagent No. VII) and 50 cc. of distilled water. The flask is placed on a wire gauze and warmed. When the liquid has commenced boiling, 25 cc. of the proteïd solution is run in, the liquid being shaken and kept boiling for two or three minutes, at the end of which, if not all the iron has been thrown down, a sufficient quantity of a di-sodium phosphate solution (Reagent No. VI) is added and the boiling continued some minutes. If it proves necessary to use more than 20 cc. of Reagent No. VI to get precipitated the whole quantity of iron, the quantitative ferric acetate precipitation is not quite to be relied upon. The precipitate is collected on a filter and washed twice with boiling distilled water, the flask being also rinsed with boiling water.

Uranium acetate precipitation. A mixture of 25 cc. of proteïd solution and an equal amount of uranium acetate solution (Reagent No. VIII) is warmed to a temperature between 50° and 70°, and left to stand in a dark place at the ordinary temperature till the next day, when the precipitate is collected on a filter and the flask and sediment washed twice with the non-dilute precipitant (Reagent No. VIII). For each 100 cc. of filtrate and washing liquid it is necessary to introduce a correction of 0.1 cc. of $^1/_{10}$ normal acid, corresponding with the solubility of the proteïd precipitation in the precipitation liquid.

Saturation Precipitation with Magnesium Sulphate. To 25 cc. of proteïd solution are added 10 drops of dilute sulphuric acid (Reagent No. X) and 25 gr. of cristallised pure mag-

[1]) Owing to the circumstance that the conditions of precipitation are somewhat altered when the proteïd solution to be examined does not contain any noticeable amount of mineral substances, it is advisable to use a solution of sublimate in quantity at least twice as large, by volume, as that of the proteïd solution, and to wash with the precipitant itself.

nesium sulphate (Reagent No. IX)[1]). The mixture is warmed, say in a warm water-bath, to the neighbourhood of 35° and kept at this temperature for an hour or so, the flask being repeatedly shaken vigorously in order to the complete dissolution of the magnesium sulphate. Afterwards, the proteïd matters thus salted out are collected on a filter which has previously been thoroughly moistened with a saturated solution of magnesium sulphate (Reagent Nr. XI). Finally, the precipitate together with the flask are washed twice with the same solution (Reagent No. XI).

Besides the above determinations (precipitations), the total amount of nitrogen in the substance under examination, as well as the amount of soluble and insoluble nitrogen, may also be estimated. Finally, it is also possible to determine the proportion of nitrogen present in the form of ammonia, which can be done by distilling a certain amount of the proteïd solution *in vacuo* with a convenient quantity of magnesium oxide, after the manner mentioned by A. Longi[2]), E. Winterstein and W. Bissegger[3]).

As regards the relation existing between the above five proteïd precipitations, it may only just be mentioned that:

The precipitation with		Contains the Proteïds
Stannous chloride = a	—	Albumin I (Leucosin).
Mercuric chloride = b	—	Albumin I (Leucosin). Albumin II (Edestin). Denucleïn.
Ferric acetate = c	—	Albumin I (Leucosin). Albumin II (Edestin). Denucleïn. Proteoses (Albumoses).

[1]) The amount of dilute sulphuric acid to be added to the proteïd solution is given on p. 50. The quantity of cristallised magnesium sulphate must be regulated by that of the proteïd solution, — always 1 gr. of $MgSO_4$, $7\,H_2O$ to each cc. of proteïd solution.

[2]) Landw. Versuchsst. **32**, 15 (1886).

[3]) Zeitschr. f. physiol. Ch. **47**, 28 (1906).

Uranium acetate = d — { Albumin I (Leucosin). Albumin II (Edestin). Denucleïn. Proteoses (Albumoses). Peptone (real Peptone).

Magnesium sulphate = e — { Albumin I (Leucosin). Albumin II (Edestin). Proteoses (Albumoses).

Designating the estimated amount of

Insoluble nitrogen ..... by Q,
Soluble nitrogen ..... by N,
Nitrogen in the form of Ammonia.. by G,

we now have all the necessary data for calculating the amounts of the various forms of nitrogen: —

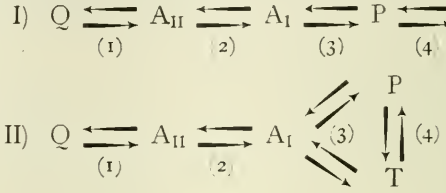
Determination	Nitrogen in the form of	Marked	
Q	= insoluble combinations	— Q	
N	= soluble combinations	— N	
a	= Albumin I	— A_I	
$b \div [a + (c \div e)]$	= Albumin II	— A_{II}	
$c \div e$	= Denucleïn	— D	= P
$c \div b$	= Proteose	— E	
$d \div c$	= Pepton	— F	
G	= Ammonia	— G	= T
$N \div (d + G)$	= Amine-Amide	— H	

The value P represents the amount of peptic, and T that of tryptic cleavage products, P + T thus corresponding with the total amount of proteolytic cleavage products.

If by the letters Q, N, A_I, A_{II}, P and T we designate the quantities of nitrogen in the above-mentioned forms as contained in the material with which a series of experiments starts, and if by the corresponding »marked« letters Q', N' and so on are designated the quantities of the same forms of nitrogen in the experimental material under examination, we shall be able to calculate the numerical values of the various transformation processes, provided that it is possible to state the relations existing between these in their consecutive order.

In order to obtain a fairly complete view of the relations of the transformations of proteïd substances, it must first be borne in mind that building up and breaking down are reverse, or reciprocal, processes.

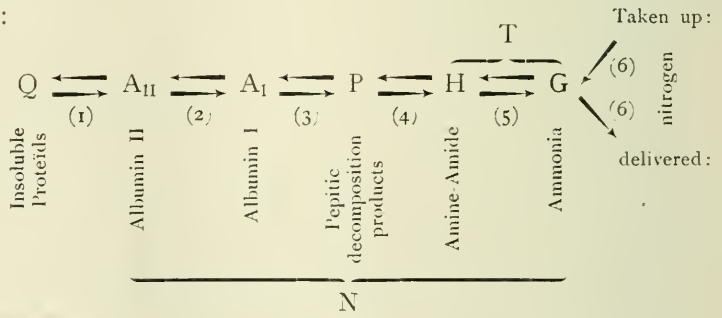
Furthermore, from the explanations given in one of my principal memoirs[1]), it is evident that the mutual relation between the processes may be expressed by one of the following two schemes[2]): —



The former scheme enables us to calculate the numerical values of the processes (1) = proteid dissolution, (2) = albumin conversion, (3) = peptic action, and (4) = tryptic action; while according to (II) we can calculate the values of processes (1) = proteïd dissolution, (2) = albumin conversion, and (3) = aggregate proteolysis. It is to be noted, however, that the value of process (3) will be exactly the same whether calculated from (I) or (II).

It cannot be established with certainty, which of the two schemes represents the true solution; but at any rate the manner in which the building up (condensation) of proteïd substances takes place is strong evidence in support of the correctness of hypothesis (I), which I have therefore adopted as the basis of the following considerations.

Our synopsis of the positive and negative proteïd transformations may now been enlarged so as to give the following groundplan:



[1]) Compt. rend. des travaux du Laborat. de Carlsberg **9**, 276—280 (1913).

[2]) For the meaning of the letters, see page 53.

The arrows ←— (positive direction) relate to the proteïd condensation, —→ (negative direction) to proteïd decomposition.

According to this ground-plan the processes may be summarised thus:

Process	Condensation		Decomposition
(1) =	Proteïd condensation	or	Proteïd dissolution.
(2) =	Albumin condensation	-	Albumin conversion.
(3) =	Peptic condensation	-	Peptic action (cleavage).
(4) =	Tryptic condensation	-	Tryptic action (cleavage).
(5) =	Ammonia condensation	-	Ammonia liberation.
(6) =	Nitrogen taken up (through the root)	-	Nitrogen delivered (to the root).

The numerical values of the different processes can be calculated, on the basis of our analytical results, from the following equations:

Process	Condensation ←—		Decomposition —→
(6) =	$(N' + Q') \div (N + Q)$	or	$(N + Q) \div (N' + Q')$.
(5) =	$(6) + G \div G'$	-	$(6) + G' \div G$.
(4) =	$(6) + T \div T'$	-	$(3) + P \div P'$.
(3) =	$(4) + P \div P'$	-	$(2) + A_I \div A_I'$.
(2) =	$(3) + A_I \div A_I'$	-	$(1) + A_{II} \div A_{II}'$.
(1) =	$Q' \div Q$	-	$Q \div Q'$.

The numbers in brackets represent the numerical values of the transformation processes concerned (see the arrows), while the not marked letters signify the analytical numbers resulting from the examination of the initial materials, and the marked letters the corresponding figures relating to the examination of the experimental materials (development and ripening samples of barley, germination and kiln-drying samples of malt, etc.).

The Development, Ripening and Storage of the Barley Grain.[1])

In order to inquire into the development and ripening of the grain, I undertook three experiments, namely: —

I. Year 1901. Prentice barley cultivated in a lot of ground

[1]) Compt. rend. des travaux du Laborat. de Carlsberg **6**, 229 (1906).

TABLE I.

Experiment No. Year (Period)	Date when the sample was taken and examined	Column marks / Stage of maturity				A_I	A_{II}	P	T	N	Q	N + Q
			10,000 corns contain									
			gr. of dry matter	gr. of ash	Acid ∞[1]) cc. of normal NaOH	— gr. of nitrogen in the form of						
						Albumin		cleavage products		soluble	insoluble	total nitrogen
						I	II	peptic	tryptic	combinations		
I 1901 (short)	24/7	Green-ripeness	220.4	7.0	44.2	0.34	0.11	0.13	1.24	1.82	2.44	4.26
	30/7	Late green-ripeness	334.7	10.4	48.7	0.56	0.16	0.16	0.72	1.60	3.66	5.26
	5/8	Yellow-ripeness	427.0	12.5	35.4[2])	0.62	0.17	0.20	0.41	1.40	5.32	6.72
	8/8	Late yellow-ripeness	445.9	12.5	32.7[2])	0.75	0.09	0.24	0.46	1.54	6.30	7.84
	13/8	Full maturity	448.2	13.2	57.6	0.77	0.07	0.23	0.54	1.61	6.79	8.40
	19/8	Over-ripeness	396.0	11.4	50.7	0.55	0.26	0.24	0.45	1.50	5.92	7.42
II 1903 (long)	27/7	Early green-ripeness	173.0	6.1	17.8	0.34	0	0.18	0.91	1.41	1.81	3.22
	3/8	Late green-ripeness	273.5	8.8	21.6	0.66	0.06	0.22	0.93	1.87	2.95	4.82
	10/8	Early yellow-ripeness	377.3	11.3	27.3	0.76	0.25	0.19	1.01	2.21	4.68	6.89
	17/8	Yellow-ripeness	456.7	12.2	34.9	0.84	0.22	0.24	0.65	1.95	6.35	8.30
	28/8	Full maturity	497.8	12.3	35.7	0.84	0.19	0.24	0.65	1.92	8.44	10.36
	7/9	Over-ripeness	483.0	12.8	38.7	0.54	0.33	0.27	0.65	1.79	8.78	10.57
III 1905 (normal)	17/7	Early green-ripeness	187.2	6.6	19.8	0.47	0.03	0.25	1.33	2.08	2.25	4.33
	8/8	Yellow-ripeness	473.4	12.3	36.8	0.95	0.19	0.24	0.74	2.12	9.02	11.14
	14/8	Full maturity	466.8	12.7	38.8	0.60	0.16	0.22	0.62	1.60	9.85	11.45
	24/8	Over-ripeness	409.7	11.2	40.9	0.60	0.19	0.25	0.59	1.63	8.77	10.40
Analytical errors — about			3.7	0.7	3.9	0.03				0.06	0.61	0.64

[1]) Here and in the sequel, the sign ∞ means »corresponding to«.

[2]) These two determinations are obviously a little too small.

belonging to the Royal College of Agriculture at Copenhagen. The period of development and ripening was of short duration, in consequence of a hot and dry summer.

II. Year 1903. Prentice barley grown in the same plot of land. Long developmental and ripening period, in consequence of the summer being cold and wet.

III. Year 1905. Primus barley from Svalöf grown in a garden at Glostrup near Copenhagen. Fairly normal developmental and ripening period, corresponding with a mean summer.

The experimental results are brought together in Table I.

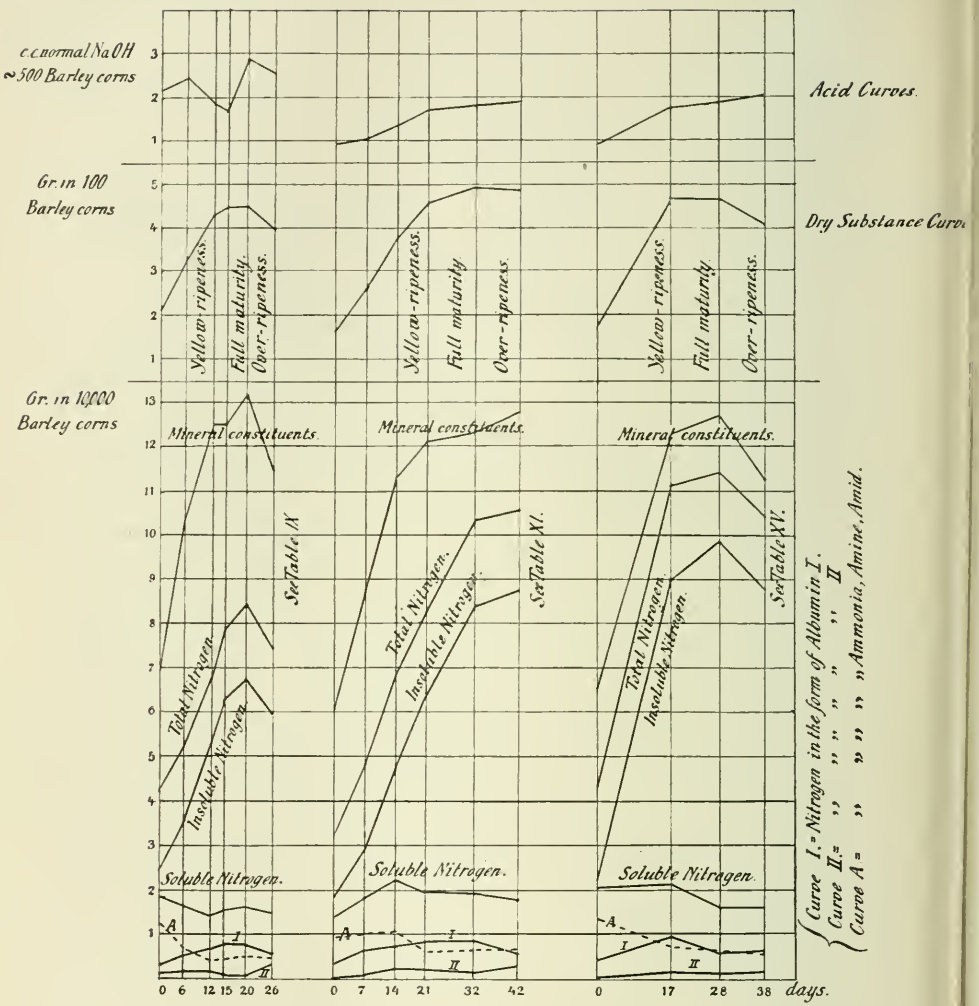
They are also plotted out on a system of coordinates, — see Curve Table 1, where, however, the Dry Substance Curve has been drawn with only $^1/_{100}$ of the value given in Table I, and the Acid Curve with $^1/_{20}$ of the real value.

The development of the barley grain consists of two stages, namely the Developmental Period properly so called, which extends to the stage of yellow ripeness, and the Ripening Period, extending to what we call the full-maturity stage. At the close of the developmental period the barley grain has reached its maximum volume, at the end of the ripening period its highest amount of dry matter. At the moment when the grain has reached the stage of full maturity, we have — allowance being made for analytical errors — reached the maximum values for dry matter, ash, acid, total and insoluble nitrogen. If the corn remains on the mother-plant beyond full maturity, these four values will again decrease in a greater or less degree, and hence it appears that the period of over-ripening is a reflection of the developmental one.

This will become still more conspicuous if we look at Curve Table 2, which represents the four just-mentioned groups of substances in their mutual relations. That part of the curves which represents from 0.7 to 3.5 gr. of dry matter is left out; yet the table will be easily intelligible by the aid of the particulars given in it. In this Table, besides the direct values, the curves must be thought to be produced beyond the initial and, on the other hand, the final experiments; these prolongations are indicated by dotted straight lines. We can thus get some insight into the course taken during the antecedent and the after periods.

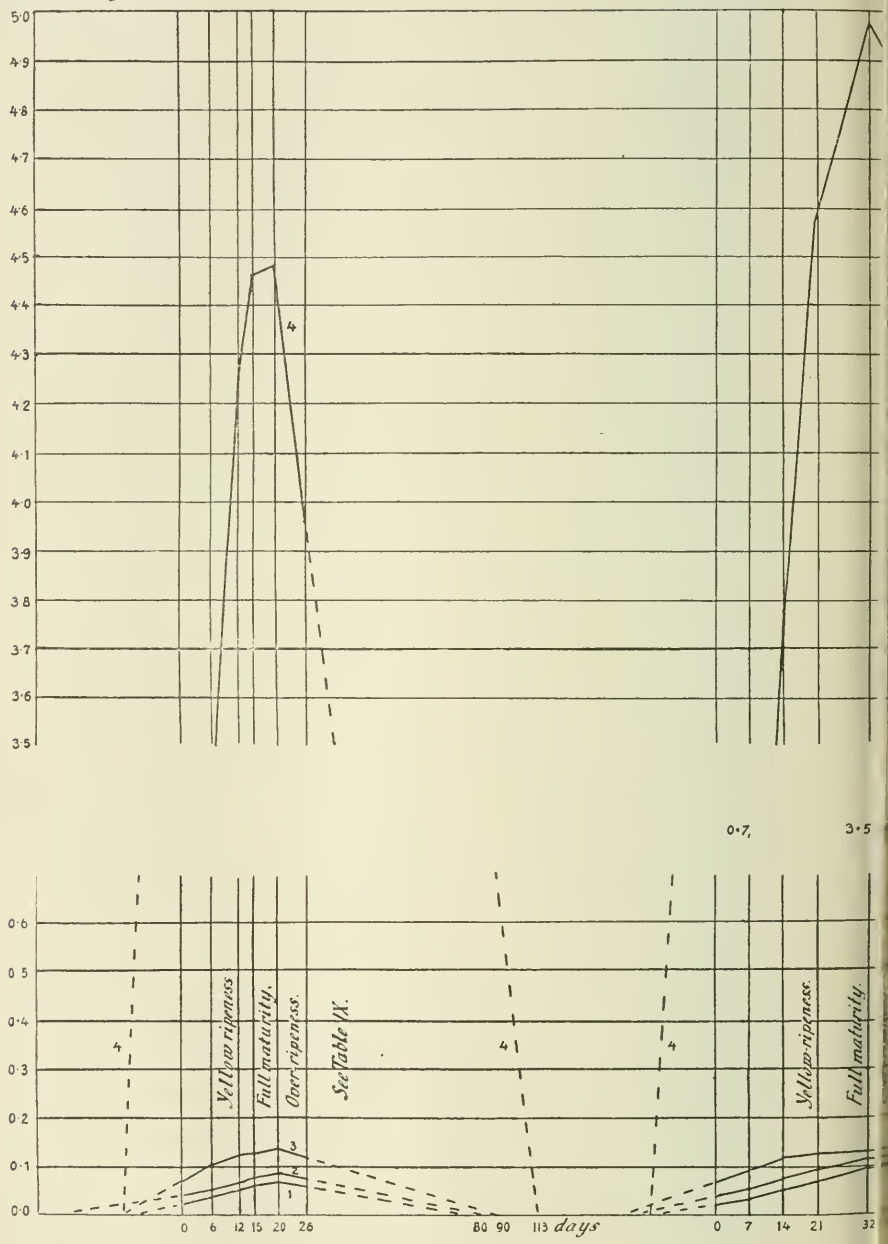
If we first examine the antecedent periods — the dotted lines in Curve Table 2 preceding the stage of green-ripeness —,

Curve Table I.



we at once meet with a pretty uniform state of things, the four curves cutting the axis of abcissas nearly in one and the same point, the distance of which from the point 0 denoting the green ripeness stage is almost equal in all three experiments. Hence the development of the barley grain from the moment of its formation till the stage of green-ripeness appears to be fairly constant despite of differences in regard to atmospheric conditions, soil, and variety of barley. Seeing now that the characteristics of the variety are implanted in the very germ of the

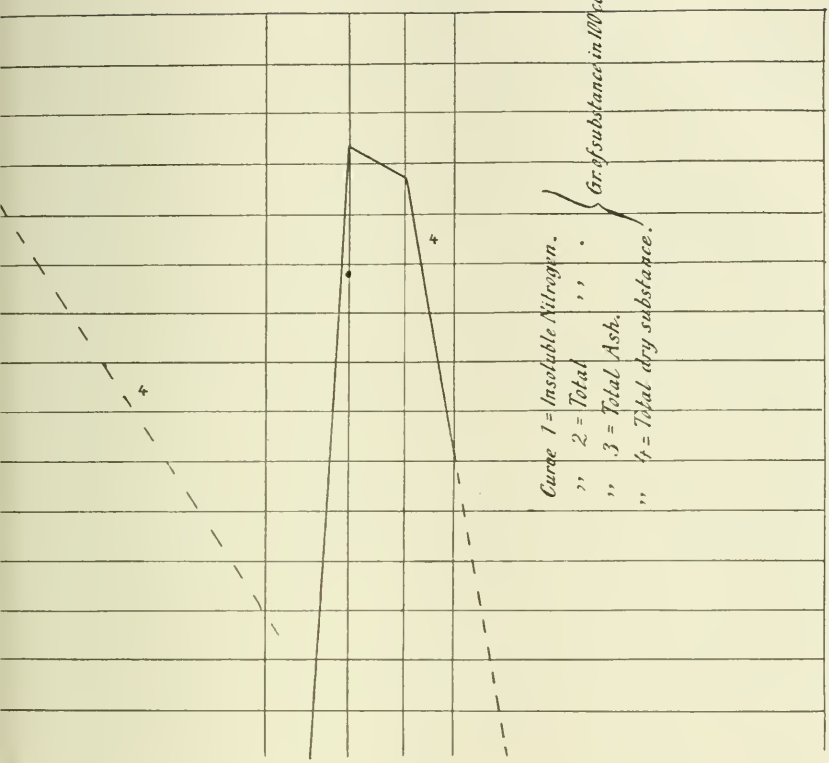
I. 1901, Short Period. II. 1903, Long Pe



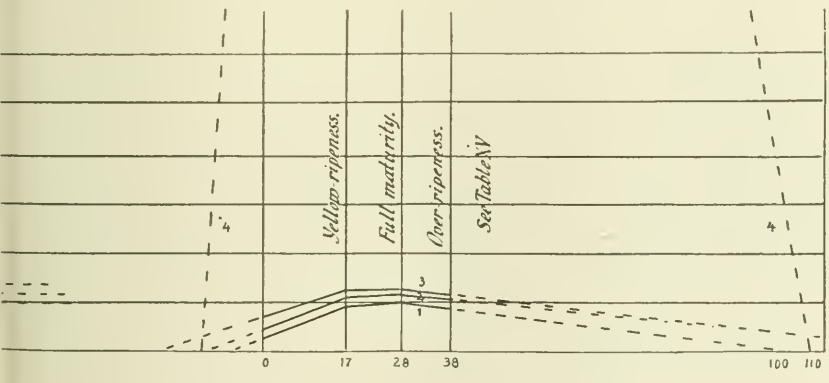
H. Schjerning.

riments.

III. 1905, Normal Period.



nce in 100 corns.

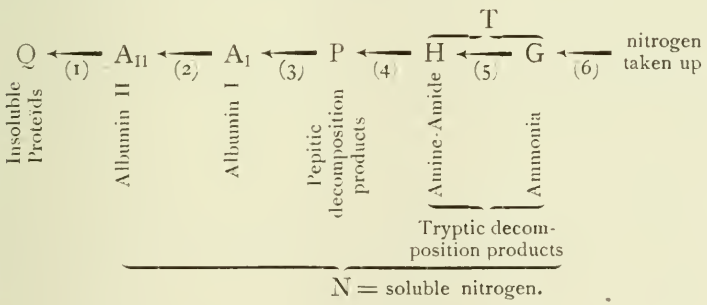


corn sown, the experimental facts referred to seem to indicate that the variety of barley cannot per se be absolutely conclusive for the composition of dry matter of the crops.

A glance at the after periods as represented on Curve Table 2 by the dotted lines continuing the stage of over-ripeness will show that barleys which have had a growth and ripening period of short duration are much more liable to suffer a loss of dry matter by over-ripening than such barleys as have had a long developmental and ripening period. That this fact is contingent upon the atmospheric conditions, is clear from the circumstance that the experiments I and II were made with the same sort of barley and grown in the same plot of land.

With regard to the purely quantitative aspect of the matter, it will be seen by Curve Table 2 that the same variety of barley yields a more large-grained crop during a long than during a short period of growth.

As far as the nitrogenous matters are concerned, I may refer to what has been established in regard to the relation between the processes determining this part of the transformation, or rather formation. It may be summarised thus:



On calculating the numerical values of the various processes of formation, we arrive at the figures set forth in Table II.

Apart from a few insignificant discrepancies, these figures enable us to define precisely what is to be understood by full-maturity: — The stage of full maturity is reached when the transformation of soluble into insoluble carbohydrates[1]), and of soluble proteïd matters into insoluble ones has ceased or reached

[1]) G. André, Compt. rend. de l'Acad. des sciences **134**, 995 (1902); **138**, 1510 and 1712 (1904); **139**, 805 (1904); **140**, 1417 (1905).

TABLE II.

Experiment No. Year (Period)	Process: Stage of maturity	Duration of the period of growth — days	Per 10,000 corns: Increase in: dry matter gr.	ash g	Acid ∞ cc. of normal NaOH	(6) supply of nitrogen	(4) tryptic condensation	(3) peptic condensation	(2) albumin condensation	(1) proteïd condensation
						Numerical value of the process expressed in gr. of nitrogen				
	Late green-ripeness	6	114.3	3.4	4.5	1.00	1.52	1.49	1.27	1.22
I	Yellow-ripeness	12	206.6	5.5	÷ 8.8*)	2.46	3.29	3.22	2.94	2.88
1901	Late yellow-ripeness	15	225.5	5.5	÷11.5*)	3.58	4.36	4.25	3.84	3.86
(short)	Full maturity	20	227.8	6.2	13.4	4.14	4.84	4.74	4.31	4.35
	Over-ripeness	26	175.6	4.4	6.5	3.16	3.94	3.83	3.62	3.48
	Late green-ripeness	7	100.5	2.7	3.8	1.60	1.58	1.54	1.22	1.14
II	Early yellow-ripeness	14	204.3	5.2	9.5	3.67	3.57	3.56	3.14	2.87
1903	Yellow-ripeness	21	283.7	6.1	17.1	5.08	5.34	5.28	4.78	4.54
(long)	Full maturity	32	324.8	6.2	17.9	7.14	7.40	7.34	6.84	6.63
	Over-ripeness	42	310.0	6.8	20.9	7.35	7.61	7.52	7.32	6.97
III	Yellow-ripeness	17	286.2	5.8	17.0	6.81	7.40	7.41	6.89	6.77
1905	Full maturity	28	279.6	6.2	19.0	7.12	7.83	7.86	7.69	7.60
(normal)	Over-ripeness	38	222.5	4.7	21.1	6.07	6.81	6.81	6.64	6.52
	Analytical errors — about		3.7	0.7	3.9			0.6		

*) These two determinations are obviously a little too small.

its maximum. The same holds good quantitatively also, both with regard to the total amount of ash and to that of free acid, only with this exception that in the case of these two groups of substances nothing is known as to the change undergone by the relation between soluble and insoluble groups or individuals.

The figures further show that, when the developmental period of the barley grain has been of short duration, the numerical value of the process of proteïd condensation (1) is always superior to that of nitrogen supply (6), whereas the case is entirely the reverse when that period is long. In a period of normal duration, on the contrary, these two processes have about the same numerical value. In other terms, when the development of the barley grain from green-ripeness to yellow-ripeness takes place during a normal period, there is a state of equilibrium between the quantity of nitrogen supplied in a given time and the nitrogen

condensed in the same time into insoluble proteïd matters; thus development and ripening go together. But in a period of short duration the condensation of soluble nitrogenous compounds into insoluble ones goes on with greater velocity than the supply of nitrogen to the grain, that is, the ripening very soon begins to prevail over the developmental process. In a long developmental period the intake of nitrogen prevails over condensation, or the ripening takes place at an extremely slow rate.

There is still another important point which can be cleared up by the aid of Table II, namely the relation between the intensities of the different processes of proteïd condensation. This question can be best answered with the help of Curve Table 3, where the abscissas mark the different processes of proteïd condensation, and the ordinates the numerical values of the different processes, expressed in gr. of nitrogen per 10,000 grains. The table contains the curves relating to all the samples of green-, yellow- and full-ripeness, with numbers denoting the duration of the experiments (from 6 to 32 days).

This curve table shows a marked difference between the experiments I (short period) and II (long period). In fact, in experiment I the process (4) is found to be $>$ (6) from "late green-ripeness" (6 days) to "full maturity" (20 days); in experiment II, on the contrary, the process (6) is $>$ (4) in the samples of "late green-ripeness" (7 days) and "early yellow-ripeness" (14 days); it is only after this latter stage that process (4) is found to be $>$ (6), and even then with an appreciably smaller difference between the numerical values than in experiment I.

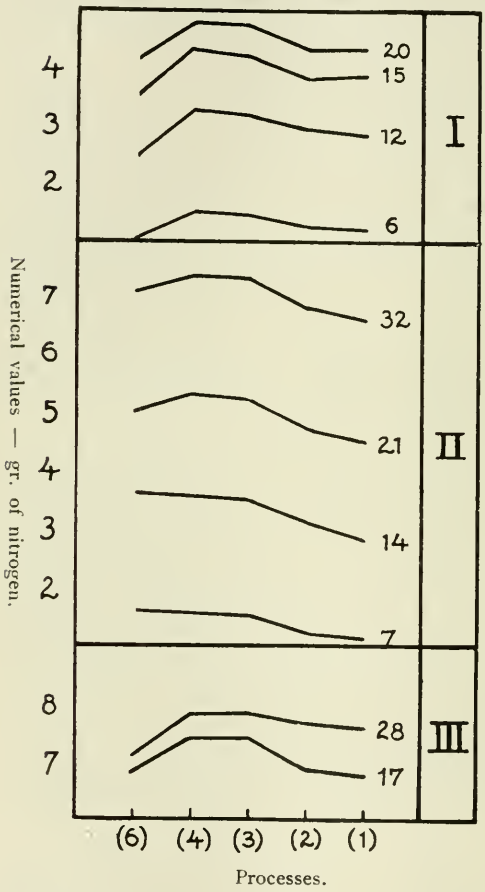
Thus, in a short period of development the tryptic condensation takes place with greater intensity than during a development of long duration.

On the other hand, during the ripening period the intake of nitrogen (6) always shows a lower numerical value than the tryptic condensation process (4); the values thereafter further decrease, quite inconsiderably in the case of peptic condensation (3), a little more in albumin condensation (2) and proteïd condensation (1). With regard to the last-mentioned process, however, we notice a difference between experiments I and II, inasmuch as, when the ripening process prevails over the developmental process — that is, from yellow-ripeness —, then proteïd condensation (1) shows a somewhat greater intensity than albu-

bumin condensation (2) in experiment I, whilst in experiment II process (2) is invariably found to be $>$ (1).

Hence it clearly appears that climatic conditions exert a noticeable influence on nitrogen condensation in the course of the development and ripening of the barley grain: — A warm and dry summer generally gives a more complete nitrogen condensation than a cold and wet summer, and this particularly in regard to condensation process (1), by which albumin II is converted into a proteïd individual insoluble in water.

Curve Table 3.



It will also be interesting to consider our experimental results as relating to different periods, with a view to ascertaining at what moment each condensation process has its maximum value. Table III is calculated to show this.

These figures prove that in each of our three experiments the maximum values of the various building-up processes occur between green- and yellow-ripeness, but nearer the latter.

This result agrees perfectly with the distinction which I have shown to exist between a developmental and a ripening period, the former period coming to a close when the stage of yellow-ripeness is over, and the latter when full-maturity is reached. But the table also shows that the transition between the developmental and ripening periods almost disappears when the growth

TABLE III.

Experiment No. Year (Period)	Process: Duration of the period of growth			Per 10.000 corns: Increase in			(6) Numerical value of process expressed in gr. of nitrogen	(4)	(3)	(2)	(1)
	from	to	days	dry matter gr.	ash gr.	acid ∞ cc. of n. NaOH	supply of nitrogen	tryptic condensation	peptic condensation	albumin condensation	proteid condensation
I 1901 (short)	Green-ripeness	Late green-ripeness	6	114.3	3.4	4.5	1.00	1.52	1.49	1.27	1.22
	Late green-ripeness	Yellow-ripeness	6	92.3	2.1	÷ 13.3*)	1.46	1.77	1.73	1.67	1.66
	Yellow-ripeness	Late yellow-ripeness	3	18.9	0	÷ 2.7*)	1.12	1.07	1.03	0.90	0.98
	Late yellow-ripeness	Full maturity	5	2.3	0.7	24.9	0.56	0.48	0.49	0.47	0.49
	Full maturity	Over-ripeness	6	÷ 52.2	÷ 1.8	÷ 6.9	÷ 0.98	÷ 0.90	÷ 0.91	÷ 0.69	÷ 0.87
II 1903 (long)	Early green-ripeness	Late green-ripeness	7	100.5	2.7	3.8	1.60	1.58	1.54	1.22	1.14
	Late green-ripeness	Early yellow-ripeness	7	103.8	2.5	5.7	2.07	1.99	2.02	1.92	1.73
	Early yellow-ripeness	Yellow-ripeness	7	79.4	0.9	7.6	1.41	1.77	1.72	1.64	1.67
	Yellow-ripeness	Full maturity	11	41.1	0.1	0.8	2.06	2.06	2.06	2.06	2.09
	Full maturity	Over-ripeness	10	÷ 14.8	0.6	3.0	0.21	0.21	0.18	0.48	0.34
III 1905 (normal)	Early green-ripeness	Yellow-ripeness	17	286.2	5.8	17.0	6.81	7.40	7.41	6.89	6.77
	Yellow-ripeness	Full maturity	11	÷ 6.6	0.4	2.0	0.31	0.43	0.45	0.80	0.83
	Full maturity	Over-ripeness	10	÷ 57.1	÷ 1.5	2.1	÷ 1.05	÷ 1.02	÷ 1.05	÷ 1.05	÷ 1.08
		Analytical errors — about		3.7	0.7	3.9	0.6				

All figures without signs are positive.

*) These two determinations are obviou ly a little too small.

periods are particularly long; for in this case all the nitrogen condensation processes are found to have practically constant values from "late green-ripeness" to "full-maturity". Thus, here again the influence of atmospheric conditions becomes apparent.

In experiments I and III the different processes are seen to have negative signs during the period extending from full-maturity to over-ripeness; the same remark applies in some measure to experiment II. This evidently means that the condensation processes have changed into decomposition or demolition processes. In the course of over-ripening there occurs a displacement of substances, which are conveyed from the seed to the other parts of the plant, that is, exactly the reverse of the process taking place during the growth period. This phenomenon surely does not, at all events not exclusively, admit of the explanation advanced by me formerly, viz. the assumption that in the stage of over-maturity respiration prevails over assimilation.

In the course of the developmental period of the barley grain (extending from green-ripeness to yellow-ripeness) the process (1) is constantly found to have a lower numerical value than the one exhibited by process (2), whereas during the ripening properly so called (from yellow-ripeness to full-maturity) (1) is superior to (2). Hence it may be inferred that the proportion of albumin II in a barley grain increases more or less during the period of development, but decreases, or at any rate remains constant while the corn is ripening. The proportion of albumin II again increases more or less during the period of over-ripeness. A climatic influence cannot be shown to assert itself in this connection.

The above-mentioned barley samples were also subjected to a set of storage experiments. They were stored in a dark and dry place at the ordinary temperature of a room, and with free access of atmospheric air. The results will be seen in Table IV. The numerical values are calculated with regard to the unity 1000 gr. of dry matter, chiefly for practical reasons. It is to be noted, however, that the dry matter weight of 10.000 corns has been incorporated in the table for the purpose of showing that, providing the barley has reached a certain degree of maturity before being reaped, and providing it be stored suitably, there

Experiment No. Year (Period)	Column marks: Stage of maturity	Stored — days	gr. of dry matter in 10,000 corns	1000 gr. of barley dry matter contains: gr. of ash	Acid ∞ cc. of normal NaOH	A I	A II	D	E	F	P	T	N	Q	N+Q	% Germinating: energy	% Germinating: capacity
						— gr. of nitrogen in the form of: Albumin I	Albumin II	Denucleïn	Proteose	Peptone	Cleavage products: peptic	Cleavage products: tryptic	soluble combinations	insoluble combinations	total nitrogen		
I 1901 (short)	Over-ripeness	0	396.0	28.8	128.0	1.39	0.66	0.28	0	0.33	0.61	1.13	3.79	14.95	18.74	—	—
		14	392.1	27.3	100.7	0.92	0.99	0.31	0	0.23	0.54	1.04	3.49	16.35	19.84	—	—
		44	400.2	25 7	105.7	0.97	0.97	0.32	0.07	0.42	0.81	1.07	3.82	14.55	18.37	—	—
		64	376.9	27.3	131.9	1.03	0.82	0.24	0.11	0.37	0.72	0.98	3.55	17.99	21.54	—	—
II 1903 (long)	Yellow-ripeness	0	456.7	26.7	76.4	1.84	0.48	0.28	0	0.24	0.52	1.43	4.27	13.90	18.17	—	—
		28	456.6	27.9	67.9	1.07	0.83	0.28	0	0.20	0.48	0.88	3.26	15.38	18.64	5.8	22.2
		56	455.7	28.2	70.2	1.14	0.83	0.22	0	0.20	0.42	0.95	3.34	15.22	18.56	63.6	75.8
	Full maturity	0	497.8	24.7	71.7	1.69	0.38	0.22	0	0.26	0.48	1.31	3.86	16.95	20.81	—	—
		28	486.7	26.0	75.6	1.66	0.45	0.33	0	0.23	0.56	1.27	3.94	18.09	22.03	5.6	12.4
		56	490.7	25.5	78.9	1.26	0.90	0.26	0	0.22	0.48	1.23	3.87	18.95	22.82	95.6	99.6
	Over-ripeness	0	483.0	26.6	80.1	1.12	0.68	0.23	0	0.33	0.56	1.35	3.71	18.17	21.88	—	—
		28	488.0	26.8	83.2	1.23	0.98	0.23	0.06	0.39	0.68	1.11	4.00	18.58	22.53	23.6	38.2
		56	482.9	27.0	86.1	1.64	0.60	0.23	0	0.39	0.62	1.41	4.27	18.65	22.92	93.6	99.4
III 1905 (normal)	Yellow-ripeness	0	473.4	26.1	77.7	2.01	0.40	0.30	0	0.21	0.51	1.56	4.48	19.05	23.53	—	—
		32	461.0	26.6	104.9	1.65	0.82	0.07	0	0.52	0.59	1.30	4.36	19.31	23.67	—	—
		60	459.2	26.8	105.4	1.72	0.65	0	0	0.52	0.52	1.31	4.20	19.97	24.17	—	—
	Full maturity	0	466.8	27.2	83.1	1.29	0.34	0.30	0	0.17	0 47	1.33	3.43	21.10	24.53	—	—
		30	464.3	26.9	100.2	1.34	0.78	0.17	0	0.28	0.45	1.18	3.75	19.90	23.65	—	—
		63	464.7	26.7	91.7	1.12	0.82	0.28	0	0.17	0.45	1.12	3.51	20.38	23.89	—	—
	Over-ripeness	0	409.7	27.4	99.8	1.46	0.46	0.34	0	0.27	0.61	1.45	3.98	21.40	25.38	—	—
		34	402.9	28.4	101.5	1.34	0.89	0.27	0	0.27	0.54	1.35	4.12	22.21	26.33	—	—
		60	406.1	27.9	100.7	1.28	1.01	0.20	0	0.32	0.52	1.43	4.24	20.83	25.07	—	—
Analytical errors — about			3.7	2.0	9.0	0.09							0.17	1.5	1.6	2.0	

is nothing that goes to show a loss of dry matter — respiration loss — to occur during storage.

In all the other columns of Table IV we almost everywhere meet with small fluctuations in one or the other direction, as far as the over- and yellow-ripeness samples are concerned, a fact which is not to be wondered at, since these samples have not reached the state of equilibrium which is observed in the stage of full-maturity. In regard to the yellow-ripeness samples the storage appears to be rather equivalent to an after-ripening, in that the proportion of tryptic decomposition products and soluble nitrogen compounds is seen to decrease and the proportion of insoluble nitrogenous matters to increase somewhat. In the over-maturity barleys the case is rather the reverse, in accordance with what has been said about the influence exerted by over-maturity in changing the various processes of proteïd condensation into decomposition processes. The fully matured barleys, on the contrary, are not influenced in either of these directions while being stored.

The albumins I and II, on the other hand, are subject to fluctuations during storage, and that at any stage of ripeness. It may therefore be established as a general rule that, if the state of equilibrium towards which the ripening barley corn is inclining has been reached before the barley is reaped, it is not disturbed during storage, only with this exception that a small quantity of albumin I is further condensed to albumin II.

Besides it appears from the table that, if the corn is reaped at an early date, the crop turns out to contain less nitrogen than it does if it is reaped later. In addition, these like many other analyses of barley go to prove that, normally, the barley corn does not contain any proteoses at all. If these occur in noticeable quantity in a barley crop, it may be considered as indicative of rather unfavourable harvesting conditions.

It has been pointed out above that tryptic condensation constantly exhibits a higher numerical value than the peptic during the development and ripening processes. Consequently, the proteoses cannot be supposed to act as intermediate terms in the condensation of amine-amide combinations into proteïd individuals of a higher order, but are exclusively to be regarded as hydrolytic cleavage products of proteïds of a higher order.

Finally, it was attempted by these investigations to elucidate

the question as to the influence exerted on the chemical composition of the barley dry matter by the species, variety and type, as also by conditions of soil and climate. I examined 45 samples of different species, varieties and types of barley, some of them grown under the same exterior conditions, whilst others came from different countries. The experimental details are given in my memoir (**6**. 291—303).

It is to be observed that, particularly with regard to the question as to the influence of soil and climate, no definite answer can be obtained until far more copious materials have been examined than those at my disposal.

Only this much can be with certainty inferred from these researches: —

The species, variety and type of barley do not in themselves affect the chemical composition of dry matter, at any rate not as far as the various groups of nitrogenous matters, mineral constituents and water-soluble acid components are concerned, whereas the cultural condition of the soil and the climatic conditions do exert some influence on the ash content of the barley dry matter and, to a certain extent, also on the amount of total nitrogen and amine-amide nitrogen, whilst the other groups of nitrogenous constituents appear to be affected in a less degree by the last-mentioned two factors than by such as the degree of maturity and duration of storage.

Transformation of the Proteïd Matters during Malting and Malt Storing.[1])

Steeping and Germination.

The first stage in the malting process is the operation which goes by the name of »Steeping«, and which consists in the barley-corn being soaked in water under suitable conditions.

The steeping process acts upon the barley grain in various ways, depending upon several factors, such as temperature, duration of the process, and degree of purity of the steeping water. We are here only concerned with the steeping time, the most important of these factors, and one which by itself is apt to affect the grain in three different directions, exerting, as it does,

[1]) Compt. rend. des travaux du Laborat. de Carlsberg **8**. 169 (1910).

an influence, first on the physiological properties or vital forces of the barley grain, secondly on the chemical composition of the grain, and finally on the transformations taking place in the course of germination.

That different barleys are differently affected by the steeping time, appears clearly from the four series of experiments recorded in Table V (**8**. 285—291).

TABLE V.

500 barley grains steeped in distilled water at 18° to 20° — hours	Barley sample							
	I		II		III		IV	
	% Germinating		% Germinating		% Germinating		% Germinating	
	energy	capacity	energy	capacity	energy	capacity	energy	capacity
6	85.0	86.8	95.2	95.6	—	—	95.6	99.4
24	86.4	88.6	94.8	95.6	94.2	97.8	—	—
48	83.6	86.8	91.8	94.0	97.6	99.0	96.8	99.2
72	81.2	88.8	74.4	79.8	89.2	97.0	—	—
96	68.6	79.6	22.0	40.0	35.6	69.2	96.8	97.8
Experim. error-ab.	2.0							

As we are here simply to deal with the influence of the steeping time in its relation to the malting process, we may confine ourselves to considering the figures expressing the percentage of germinating energy, that is, the percentage of corns that have germinated at the end of 3 days. The figures clearly show that sample I is of a rather indifferent quality as a malting object, owing to the germinating energy having been impaired. Barley of such a condition has been damaged during harvesting, or perhaps even still more during storage in the granaries. If analysed by the usual method, by which the germinating energy is estimated after only 6 hours' steeping, the samples II, III and IV will be found to be well fit for malting purposes; but on closer inspection it will be seen that sample II has reached its maximum of germinating energy already after 6 hours' steeping, but III only after 48 hours, while in the case of IV the germinating energy remains constant even at the end of 96 hours. It is obvious that the malting processes would take a very unfavourable course if the barleys II, III and IV were mixed to-

gether, say in equal quantities, and the blend steeped for 4, or only 3, days. A uniform germination would never be obtained, since the vitality of the three barleys is subject to so considerable variation. We are therefore warranted in concluding that the normal steeping of any given barley coïncides with the point of time when the sample is found to be possessed of the greatest germinative energy, and consequently also the greatest evenness, and hence it appears that a thorough inquiry into the way in which vitality is affected by the steeping time is of the greatest consequence in regard to a correct valuation of the fitness of a barley for malting purposes.

About the influence exerted by the steeping time upon the chemical composition of the barley grain (**8**. 291—295) there is not much to be said, it being quite a matter of course that the longer time the steeping process is continued, the larger a quantity of the water-soluble matters contained in the grain will be extracted. It has been established that the tryptic action comes into operation very soon (already after one day's steeping), albumin conversion (transformation of albumin II into albumin I) and peptic action without much delay. Accordingly, the steeping process may be compared to an incipient germination, and indeed it is so, providing the process is not continued so long as to destroy the vitality. Thus a certain genetic relation will be seen to exist between the chemical and the physiological aspect of the steeping process, — but only till we have attained the optimum of steeping; if the steeping is continued beyond this point, the physiological forces are weakened more speedily than are the chemical ones.

Thus, the optimum of the steeping time can be determined both physiologically and chemically, the former way being, however, both the easier and the more certain of the two.

It will be emphasised later on, as a most important fact, that different barleys may require to be steeped for different lengths of time to attain the optimum of the physiological powers.

Before going on to inquire into the question as to the way in which proteïd transformation takes place during malting, and as to the influence exerted by the different malting factors, it will be natural to premise a comparative summary of the relation between the fundamental researches published by Th. B. Osborne on the proteïd substances of barley and malt, on the

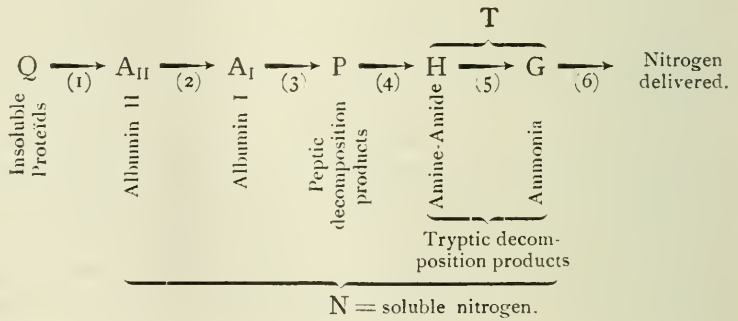
one hand, and, on the other, the results arrived at by my method of proteïd precipitation. The scheme below gives such a summary:

Marks	Q	A_{II}	A_I	P	T
Determined by Schjerning's Precipitation method in the form of	Insoluble compounds	Albumin II	Albumin I	Peptic decomposition products	Tryptic decomposition products
	Nitrogen in the form of compounds				

According to Osborne

Barley contains	insoluble in water			soluble in water		
	insoluble in alcohol and neutral salt solutions	soluble in alcohol 75 per cent	soluble in neutral salt solutions 10 per cent	soluble in neutral salt solutions 10 per cent		
	Insoluble proteïds	Hordeïn	Edestin	Edestin-salts	Leucosin	Proteoses
Malt contains		Bynin	Bynedestin			

This schedule shows the course taken by proteïd transformation; it may, however, be simplified and at the same time completed as follows:



In the course of germination the insoluble proteïd matters contained in the barley corn are partially transformed, through one or more unknown intermediate stages, into albumin II; this is what we term the Proteïd Dissolution process (1). The albumin II pre-existing in the barley corn, as well as the albumin II formed during germination from the insoluble proteïd substances, are further converted into albumin I = Albumin Conversion process (2). Albumin I is broken down by the peptase to peptic decomposition products (denucleïn + proteose + peptone) = Peptic Action (3). Finally, the peptic decomposition products are demolished by the tryptase to tryptic decomposition products (amine-amide and ammonia) = Tryptic Action (4). Of the tryptic decomposition products some are consumed in the formation of the rootlet = Process (6). As the rootlet was always removed before the examination of the malt, the amount of nitrogen spent in this way must be made allowance for as a nitrogen loss.

In order to facilitate both the description and the comprehension of the researches now to be set forth, it may be well to forestall the march of events by putting and answering the question: When is the proteïd transformation going on during malting to be considered to have come to a close statisfactory in every way?

If we bear in mind the fact that albumin conversion (2) is the proteïd transformation process which is the last to reach its completion, and further that the barley corn in itself always contains a certain small amount of nitrogen in the form of albumin II, the above question can be answered by means of a very simple equation. Indeed, if the proportion of nitrogen existing in the form of albumin II in the barley grain itself, or rather in the steeped grain, is supposed to be = a, then proteïd transformation will be entirely brought to a close in the course of malting when we have reached the state of equilibrium: Process (2) = process (1) + a.

Expressed in words, this equation means that malt in which proteïd transformation has come to a satisfactory close must not contain albumin II at all.

The justness of this demand is fully confirmed by the results obtained by Th. B. Osborne in his above-mentioned researches on the proteïd matters of barley and malt. Osborne shows that albumin II (edestin and edestin salts) must be classed with

the groups of proteïds designated as "globulins" and characterised by certain chemical pecularities, more especially by the fact that, when occurring dissolved in a more or less dilute salt solution such as for instance beer, they will be sooner or later converted into insoluble forms; thus they give rise to proteïd turbidity, and there cannot then be made too great demands in regard to the complete transformation of albumin II during the malting processes.

If during malting we have reached the stage of proteïd transformation expressed by: Process (2) = process (1) + a, the result will be that we have also Process (1) = process (3) or perhaps Process (1) a little inferior to (3). If, on the contrary, we find (1) > (3), the peptic action has been too weak. The tryptic action (4) is always noticeably lower than the peptic action (3), and we likewise find process (6) considerably lower than process (4).

Consequently, a perfectly satisfactory proteïd transformation in the malting process is reached when we find: Process (2) = process (1) + a = completed albumin conversion, and Process (1) $\leq$ process (3) = satisfactory peptic action.

This will hold good as well in the case of two-rowed as in that of six-rowed barley.

Besides the different proteïd transformation processes, it is also possible to follow the extract formation (corresponding in some measure with transformation of carbohydrates), the physiological oxidation process and rootlet growth, as also the changes which take place in mineral constituents and in the substances of acid reaction and soluble in water[1]).

The changes which the mineral constituents undergo in the course of germination and malting are, generally speaking, so inconsiderable that we need not dive into this question, but may content ourselves with exemplifying them by one particular instance (see Table VI). The fact may be expressed as follows: —

During a normally conducted germination, mineral constituents do not undergo any quantitative change beyond the conversion of insoluble into soluble ones in a quantity corresponding to that which is taken up by the root within the same space of time. Accordingly, in a normally and fully germinated malt the amount of water-soluble mineral constituents calculated in proportion to

[1]) As for the calculation of the numerical values of these processes, I refer to my original memoir, pp. 191—192.

TABLE VI.

According to Tables XX and LX in the original paper. Normal duration of steeping = 2 days.

Process								I	R	$\frac{I}{R}$	(1)	(2)	(3)	(4)	(6)	Practical valuation			
Steeped at 18° to 20° during — days	The value a[1])	Germinated at 14° — days	Per 10,000 corns													% of extract of malt dry-substance	% yield of malt of barley dry-substance	Clarification of the wort	Remarks
			Increase in		Decrease in ash			oxidation loss	rootlet loss	Quotient $\frac{I}{R}$	Numerical value of process expressed in gr. of nitrogen								
			gr. extract	Acid ≙ cc. of normal NaOH	soluble combinations gr.	insoluble combinations gr.	Sum gr.				Proteïd dissolution	Albumin conversion	Peptic action	Tryptic action	Used in rootlet growth				
1	0.17	0—4	265.0	÷1.2	÷0.15	÷0.03	÷0.18	5.5		—	1.36	1.31	1.26	0.95	0.20	74.00	96.5	rather bad	
		0—7	267.6	2.4	÷0.05	—	—	8.1	10.5	0.8	1.91	1.91	1.77	1.44	0.54	77.56	93.0	very good	
		0—10	266.6	2.6	0.14	0.29	0.43	10.5	14.3	0.7	2.09	—	1.95	1.65	0.68	78.69	91.3	excellent	
2	0.15	0—4	256.4	÷0.4	÷0.12	0.01	÷0.11	1.4	4.9	0.3	1.21	1.34	1.18	0.91	0.17	72.71	95.1	rather bad	
		0—7	259.7	3.8	—	—	0.75	15.0	12.3	1.2	2.11	2.26	2.10	1.61	0.53	78.33	89.4	good	
		0—10	248.9	6.0	0.28	0.48	0.76	25.4	20.9	1.2	2.49	2.64	2.51	1.98	0.85	79.61	84.3	excellent	Some hussars
3	0.10	0—4	215.2	÷7.7	÷0.01	÷0.43	÷0.44	—	—	—	0.21	0.15	0.16	0.09	÷0.13	59.34	—	bad	
		0—7	254.2	÷0.8	0.07	÷0.25	÷0.18	6.1	5.2	1.2	1.02	0.93	0.86	0.71	0.17	73.93	92.7	rather bad	Some hussars
		0—10	251.2	8.3	0.03	0.22	0.25	16.0	13.4	1.2	1.66	1.56	1.56	1.20	0.24	77.14	87.8	good	Many hussars and ungermin. grains
Anal. errors — about			2.6	3.3	0.1	0.3	0.2	2.4		0.1	0.13					0.6			

All figures without signs are positive.

[1]) a = gr. of nitrogen contained in 10,000 steeped grains in the form of albumin II.

total ash, will be equal to the amount existing in the barley from which the malt was prepared. —

We are now going to inquire into the influence exerted on the transformation processes taking place in germination by the different factors coming into play: Steeping Time, Germination Temperature, Germination Time, Moisture, Oxygen Tension, Light.

Influence of Steeping Time on the transformations (**8**, 295—304). The numerical values and other particulars by means of which this question may be elucidated are brought together in Table VI. It is to be borne in mind that the barley sample on which these experiments were made showed the maximum of vitality after forty-eight hours' steeping. Thus, the normal steeping time of the sample is 2 days; consequently, a duration of one day would be under-steeping, while 3 days would mean over-steeping.

If we follow the three numerical series Oxidation loss (I), Rootlet loss (R) and Quotient ($\frac{I}{R}$), it appears clearly that under-steeping does not completely set free the latent physiological powers (oxidation and rootlet growth), nor does it release them in the inter relation in which they are stored up in the barley-grain — $R > I$. Over-steeping, on the contrary, liberates the latent physiological powers in the right proportion; but both the released powers are again lessened without their inter-relation suffering any modification — $I > R$, corresponding with a normal steeping.

It will further be noticed that the powers tending to effect the conversion of carbohydrates (extract formation) come into operation readily and quickly, but are weakened somewhat in over-steeping, whereas acid-formation and velocity of growth of acrospire increase with the steeping time.

As regards the processes of proteïd transformation, it will be seen that they have their highest numerical values after two days' and their lowest values after three days' steeping; after one day's steeping we have Process (2) = process (1), and Process (1) > process (3), after two days (2) = (1) + a, and (1) = (3), and after three days (2) < (1), and (1) > (3). Thus, the result is, that normal steeping brings about a perfectly normal (completed) proteïd transformation, offering a state of equilibrium between all the processes; moreover, it gives the greatest quantitative effect. Both under- and over-steeping not only exert a weakening in-

fluence on all the proteïd transformation processes, but also modify the relations between them, so that in either case the result is a quantitatively and qualitatively incomplete transformation of the proteïds. Neither the albumin conversion nor the peptic decomposition is completed.

The important question as to the minimum limit of the normal duration of steeping has been dealt with in my memoir (**8**, 348—362), but could not be solved completely owing to the rather unsatisfactory condition of the materials worked upon. As we shall see later on, too high a degree of moisture in the barley laid out to germinate is to be avoided; but in the course of steeping the barley grain must take up the amount of water required for a normal germination, so that a further supply of water may be dispensed with. The time required for the fulfillment of this demand is at least forty-eight hours. Recent experiments have shown that, when a barley is used whose normal steeping time is less than forty-eight hours, the result will very often, if not always, be an incomplete proteïd transformation.

Hence it appears that, as a rule, barley which has reached the maximum of germinating energy already after six hours' steeping, and whose germinating energy is weakened by prolonged steeping, is not fit for malting purposes at all. A barley that reaches its maximum of germinating energy after twenty-four hours' steeping, and which is weakened by a continued steeping, must be considered as being of a questionable quality as a malting object. Only those barleys which do not attain their full vitality till after a forty-eight hours' or still longer steeping, may be ranked among the good malting objects.

From what has been said it appears, that a thorough inquiry into the conditions of vitality of a barley is of the greatest consequence in regard to a correct valuation of its fitness for a malting purposes. —

As regards the other germinating factors, we shall have to refer to Table VII, which gives the requisite particulars as also the values calculated for the different transformation processes.

The influence of the germination temperature will appear from the experimental series I and II in this table (**8**, 306—313).

The aggregate transformation of carbohydrates, which is conditioned by the three factors extract, oxidation loss and rootlet loss, increases as the germination temperature rises. The

TABL

The same sample of barley for all tl

Experimental series		Process: Germination after normal steeping (48 hours) Number of days	Temperature	Conditions of moisture	Air-renewal	Conditions of light	Increase in gr. extract	Increase in Acid ≈ cc. of normal NaOH	I Oxidation loss	R Rootlet loss	$\frac{I}{R}$ Quotient
											Per 10,0
I		0—4	14°	normal	Excess of air	Darkness	256.4	÷0.4	1.4	4.9	0.3
		0—7					259.7	3.8	15.0	12.3	1.2
		0—10					248.9	6.0	25.4	20.9	1.2
		0—12					248.5	6.4	21.0	23.6	0.9
II	A	0—4	16°	normal	Excess of air	Darkness	259.9	÷0.5	9.9		—
		0—7					251.5	6.0	38.1		—
		0—10					246.2	7.5	25.6	22.7	1.1
	B	0—4	20°				260.2	1.1	13.8	12.0	1.2
		0—7					244.5	2.1	26.0	19.7	1.3
		0—10					233.1	3.2	37.9	23.8	1.6
		0—12					231.7	4.4	40.0	22.0	1.8
	C	0—4	26°				243.0	÷1.1	19.1	14.6	1.3
		0—7					232.1	1.6	28.0	19.0	1.5
		0—10					222.0	1.7	41.1	18.8	2.2
III	A	0—4	14°	under normal	Excess of air	Darkness	236.3	3.7	0.1	2.5	—
		0—7					265.1	2.9	6.8	9.6	0.7
		0—10					256.5	6.9	13.3	16.1	0.8
	B	0—4		over normal			244.0	÷2.6	2.6		—
		0—7					259.6	2.5	22.3		—
		0—10					252.7	9.4	18.7	15.9	1.2
IV	A	0—4	14°	normal	Deficit of air	Darkness	223.4	÷4.3	0.7	1.5	0.5
		0—7					254.6	÷3.1	3.1	6.4	0.5
		0—10					252.7	0.7	13.4	11.4	1.2
	B	0—4			suitable quantity of air		235.9	÷5.1	0.1	3.2	—
		0—7					263.0	2.4	4.1	9.7	0.4
		0—10					250.5	3.0	21.8	15.1	1.4
V	A	0—4	16°	normal	Excess of air	Dispersed daylight	257.2	÷2.7	9.6		—
		0—7					258.7	5.9	17.7	13.4	1.3
		0—10					247.8	8.8	21.2	20.3	1.0
	B	0—4				Partial sunlight	266.2	3.9	2.0	7.3	0.3
		0—7					259.9	5.8	17.2	15.3	1.1
		0—10					250.3	6.4	20.1	21.3	0.9
Analytical errors — about							2.0	3.3	2.4		0.1

All figures withc

riments. Value $\alpha = 0.15$ gr.

(1)	(2)	(3)	(4)	(6)	Practical valuation			According to Tables — in the original paper
s								
Numerical value of process expressed in gr. of nitrogen					% of extract of malt dry substance	Clarification of the wort	Remarks	
dissolution	Albumin conversion	Peptic action	Tryptic action	Used in root-let growth				
21	1.34	1.18	0.91	0.17	72.71	rather bad		XXII and LXI
11	2.26	2.10	1.61	0.53	78.33	good		
49	2.64	2.52	1.98	0.85	79.61	excellent	A few hussars	
57	2.63	2.59	2.05	0.83	79.06	excellent	Many hussars	
31	1.45	1.23	0.92	0.15	74.48	rather bad		XXII and LXI
22	2.33	2.20	1.71	0.69	78.41	very good		
47	2.62	2.48	1.96	0.83	79.28	excellent	Some hussars	
—	—	—	—	0.46	78.11	good		
93	—	—	—	0.71	78.05	good		
19	2.34	2.23	1.76	0.97	78.42	very good	Many hussars	
32	2.33	2.30	1.86	0.95	78.05	very good	Many hussars	
57	1.60	1.49	1.22	0.71	74.71	rather bad		
71	—	—	—	0.77	74.41	good	5th day some hussars	
84	1.82	1.77	1.40	0.80	74.23	good	Many hussars	
59	0.64	0.42	0.28	0.14	66.32	rather bad		XXIV and LXV
85	1.77	1.77	1.37	0.24	77.39	good		
25	2.24	2.21	1 76	0.63	77.86	excellent	A few hussars	
56	0.48	0.51	0.38	÷0.01	65.65	rather bad		
56	1.80	1.78	1.41	0.35	77.12	good		
.1	2.43	2.44	1.95	0.76	77.91	excellent	Many hussars	
6	0.20	0.19	0.03	÷0.03	60.45	very bad	Strong ethereal odour	XXVI and LXVI
78	0.75	0.74	0.51	0.11	71.38	very bad	Pure malt smell	
68	1.69	1.59	1.28	0.43	75.65	bad	» » »	
68	1.19	1.10	0.91	0.06	65.68	very bad	Faint ethereal odour	
4	1.47	1.47	1.14	0.26	76.22	bad	Pure malt smell	
74	1.95	1.94	1.62	0.62	77.79	good	» » » A few hussars	
5	1.55	1.36	1.12	0.40	76.63	rather bad		XXVIII and LXVII
1	2.35	2.25	1.81	0.74	78.92	good	Some hussars	
0	2.44	2.29	1.84	0.63	78.08	excellent	Many hussars	
4	1.79	1.66	1.27	0.18	76.15	bad		
4	—	—	—	0.62	79.62	very good	Some hussars	
9	2.38	2.37	1.91	0.75	78.85	very good	Many hussars	
		0.13			0.6			

are positive.

optimum lies in the neighbourhood of 20°. Beyond this temperature there is a falling-off in carbohydrate transformation. The velocity of reaction is greatest in the first 4 days of germination, rising with the temperature up to 20°. At higher temperatures than this, the velocity diminishes, so that the time of reaction must be prolonged if a definite transformation has to be accomplished.

The physiological oxidation process (I) increases with the temperature up to 26°, or probably even some degrees higher. The velocity also increases at least until 26°.

The root-growth (R) increases with the temperature until the optimum about 20° is reached. The initial velocity of growth likewise increases, but in the first 4 days up to 26°, if not beyond.

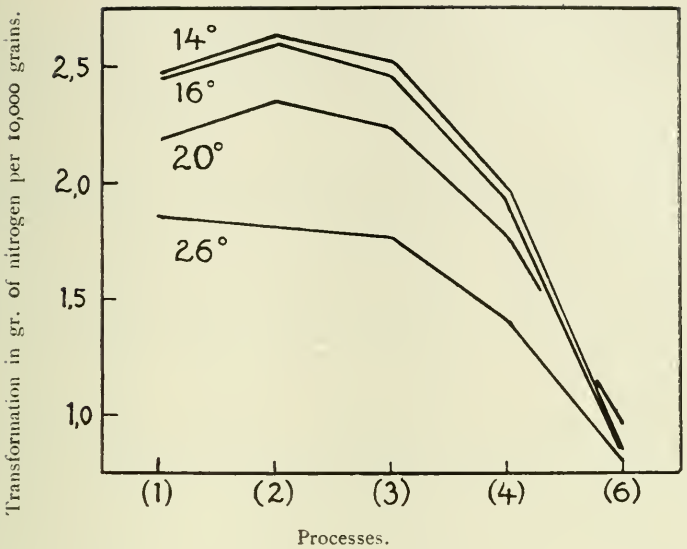
As the optimum of the oxidation process is at about 26° and that of root-growth at about 20°, it follows that the quotient $\frac{I}{R}$ will be noticeably affected — augmented — by temperatures higher than 20°.

The optimum of acid-formation lies in the vicinity of 16°.

The way in which proteïd transformation is influenced by the malting temperature is elucidated by Curve Table 4, where the abscissas represent the different transformation processes, and the ordinates their numerical values, expressed, as usual, in grammes of nitrogen per 10,000 grains. These curves give a clear picture of the quantitative relations of the different processes of proteïd transformation, and at the same time show the quantitative influence of temperature on each process.

The numerical values are those obtained after 10 days' germination. The greatest action is seen to be attained at 14°, and nearly the same values are exhibited at 16°. But beyond this temperature they are lessened considerably as the temperature rises, this being true particularly in regard to the processes of proteïd dissolution (1) and albumin conversion (2), whilst the peptic action (3) is seen to decrease somewhat less, tryptic action (4) still less, and root-formation (6) only very slightly. Everything goes to show the maximum effect to be produced at 14° or perhaps 13°; but the quantitative effect is almost the same when germination is conducted at a temperature comprised between 13° and 17°. These two temperatures are the limits of the optimum zone; if they are exceeded, the whole of the proteïd transformation is weakened quantitatively. The relations between the

Curve Table 4.



individual transformations, however, are not changed until the temperature exceeds 20°. In fact, a glance at Curve Table 4 will show that at 20° the relations between the five processes are exactly the same as it is at 16° and 14°. But at 26° the state of affairs is somewhat altered, the albumin conversion (2) and peptic action (3) being lessened more than proteïd dissolution (1).

This state of things may be expressed by the following equations: —

Germination at	After 10 days:
14 — 16 — 20°	Process (2) = process (1) + a. Process (1) $\leqq$ process (3).
26°	Process (2) < process (1) + a. Process (1) > process (3).

Thus, at 14, 16 and 20° we have a perfectly normal and completed proteïd transformation, whereas at 26° we meet with an abnormal transformation, in so far as albumin conversion (2) and peptic action (3) have not been completed.

We have thus seen that the optimum temperature of a normal proteïd transformation completed in the course of germination, is comprised between about 13° and about 17°. If germination is conducted between 17° and 20°, the result will be a quanti-

tative weakening of proteïd transformation, whilst the relations between the different transformation processes remain unaffected. At a germination temperature higher than 20° both the quantitative and the qualitative aspect are influenced, the result being an abnormal transformation, — the malt will always contain albumin II.

Experiments have shown that care must be taken to prevent the temperature of germinating barley from going down below the minimum limit of the optimum zone (about 13°), because otherwise the root-growth will be noticeably impaired (**8**, 339—348).

It may be noted in this connection that the velocity of development of the acrospire increases with the temperature of germination. —

Some insight into the influence exerted by the germinating time upon the transformations may be gained from the experiment series I and II B in Table VII, or — since that influence with regard to the carbohydrates has been dealt with previously — from the annexed Table VIII, which gives a view of the velocities of reaction *per diem* in the different periods (**8**, 313—321).

TABLE VIII.

Germinated			Velocity of reaction per day for process						
			I	R	(1)	(2)	(3)	(4)	(6)
			expressed in gr. nitrogen						
at temperature	intervals	days	Oxidation loss	Rootlet loss	Proteïd dissolution	Albumin conversion	Peptic action	Tryptic action	Used in rootlet growth
14°	0—4	4	0.35	1.23	0.32	0.34	0.30	0.23	0.04
	4—7	+ 3	4.53	2.47	0.30	0.31	0.31	0.23	0.12
	7—10	+ 3	3.47	2.87	0.13	0.13	0.14	0.12	0.11
	10—12	+ 2	0	1.35	0.04	0	0.04	0.04	0
Mean velocity after 10 days' germination			2.54	2.09	0.25	0.26	0.25	0.20	0.09
20°	0—4	4	3.45	3.00	0.28	0.22	0.22	0.18	0.12
	4—7	+ 3	4.07	2.57					0.08
	7—10	+ 3	3.97	1.37	0.09				0.09
	10—12	+ 2	1.02	0	0.07	0.04	0.04	0.05	0
Mean velocity after 10 days' germination			3.79	2.38	0.22	0.22	0.22	0.18	0.10

It will at once be noted, as a general result, that in regard both to carbohydrates and proteïds, the greatest amount of decomposition and transformation is, as a rule, effected in the first four days of germination.

In respect of the vital forces the greatest velocity of reaction is met with a little later, generally between the 4th and 7th day of germination. As was pointed out in what precedes, the velocities are more or less influenced by the temperature of germination, by which the time of germination must therefore be regulated.

The question which much necessarily first be cleared up in this connection, is that relating to the stage at which the transformations are to be looked upon as completed.

By following the mutual relations of the different processes of transformation, as set forth in the experimental series I in Table VII, it will soon become conspicuous that in the course of a germination extending from 0 to 10 days we throughout meet this state of equilibrium:

Process (2) = process (1) + a, and Process (3) $\geqq$ process (1), that is to say, a completed albumin conversion and satisfactory peptic action. But at the end of 12 days the state of things is found to have undergone some alteration, namely thus:

Process (2) $<$ process (1) + a, and Process (3) $\geqq$ process (1), that is, an incomplete albumin conversion, but a satisfactory peptic action.

An inspection of the series II A and B will lead to the same result. But the series II C shows:

Process (2) $<$ process (1) + a, and Process (3) $<$ process (1), consequently an incomplete albumin conversion and also an incomplete peptic action. In this experiment, however, the maximum limit of the optimum temperature zone has been exceeded to a considerable extent.

It will be observed that in the two series I and II B many "hussars" have developed after 12 days' germination. Accordingly we may naturally distinguish between two stages, namely normal germination and over-germination.

The foregoing considerations may be summed up as follows: —

Normal germination is completed when proteïd transformation has advanced to:

Process (2) = process (1) + a, and Process (3) $\geqq$ process (1).

This is the moment when the extract yield of malt dry substance has reached the highest percentage. Consequently, a really satisfactory conversion is not obtained until normal germination is at an end, since albumin conversion is at this moment completed and peptic action satisfactory.

Over-germination causes some of the forces operating in normal germination — rootlet-growth, physiological oxidation, albumin conversion and peptic action — to suspend their action entirely or partially; one process (transformation of carbohydrates) even begins to operate in the opposite direction. Only the tryptic action and acid-formation go on in the same manner and in the same direction as in normal germination.

The influence exerted by the conditions of moisture (**8**, 325—330) on the chemical changes which take place in the germinating barley corn is shown by Table VII, series I (normal conditions of moisture[1]), III A (under-normal moisture[2]) and III B (over-normal moisture[3]).

It will be noticed at once that the extract yield is reduced when germination is conducted under abnormal conditions of moisture; in other words, the transformation of carbohydrates is impaired both by too dry a germination and by one that is too wet. The two vital forces are also influenced by the conditions of moisture, but in a somewhat different way: The process of oxidation is impaired more than the rootlet-growth by too dry a germination, whereas both processes are equally weakened when germination is conducted with an excess of moisture. The development of the acrospire, on the contrary, is favoured by too high a degree of moisture. The acid-formation appears to proceed more slowly under abnormal than under normal conditions of moisture.

As regards the processes of proteïd transformation, they are quantitatively weakened by too dry a germination, whilst in the case of excess of moisture they are only retarded, but not really

[1]) The barley was steeped forty-eight hours and subsequently germinated with supply of air which had been saturated with water-vapours at the temperature of the experiment.

[2]) Same steeping time (48 hours). Germination with supply of air dehydrated in the presence of concentrated sulphuric acid.

[3]) Same steeping time. The air supplied during germination had been saturated with water-vapours, and a little water was also sprayed over the germinating corns by means of a pulverisator.

weakened. On the other hand, the relations between these processes are somewhat altered both when the degree of moisture is too low and when it is too high. In fact, we find in either case Process (2) $<$ process (1) $+ a$, and generally also Process (3) $<$ process (1). Consequently, the albumin conversion, if taking place in abnormal conditions of moisture, is not completed, and a particularly dry germination tends to impair the peptic action also.

The germination factor termed Oxygen Tension or Renewal of Air is, as might be expected, of great importance for the various transformations (**8**, 330—334). A comparison of the figures set forth in Table VII, series I, IV A and B, will immediately show that too weak an oxygen tension (insufficient supply of air) during germination is apt to impair the internal and external mechanism of the whole germination. This holds good of carbohydrate transformation, acid-formation, vital powers and all the proteïd transformations; if there is not an excess of air, all these processes are weakened. Furthermore, the relations between the different proteïd transformations will be deranged because albumin conversion and peptic action are weakened more than proteïd dissolution. This appears clearly from the fact that in the series IV A and B we constantly find Process (2) $<$ process (1) $+ a$, and Process (3) $\leq$ process (1).

It is worthy of remark that, since the relation between the oxidation process and rootlet-growth is not noticeably affected by the oxygen tension during a germination period of ten days, there appears — at any rate as far as barley is concerned — to exist a certain regular interdependence between these two processes. —

The influence of Light on the transformations taking place in germinating barley (**8**, 334—338) is brought out by the series I and V A and B in Table VII. Although the difference of intensity of this agency was in my experiments almost as great as was practicable in the series I and II B, the quantitative changes were scarcely affected to any appreciable degree. The figures seem to show that the carbohydrate transformation, oxidation process and rootlet-growth are slightly impaired by the operation of light, without the relation between oxidation and root-growth undergoing any change. The acidity is not affected by light. Certain facts appear to favour the belief that lights tends to impair the quantitative aspect of proteïd transformation. Further, it is manifest that the ratio

between proteïd dissolution and albumin conversion is more or less unfavourably altered, particularly by direct sun-light, which gives: Process (2) < process (1) + a.

As for the sample exposed to dispersed day-light, it shows the same result up to the 7th day of germination; but at the end of 10 days it has nevertheless reached a complete albumin conversion: (2) = (1) + a.

There is a slight indication that the peptic action (3) is weakened a little more than proteïd dissolution (1); yet the deviations relating to these two processes are too small to furnish conclusive evidence.

As regards the development of the acrospire, on the contrary, it has been placed beyond question that it is furthered by light.

Thus, the results are generally the same as in over-germination — an increased development of the acrospire and a weakening of albumin conversion, and the facts established in regard to the influence of light may be summed up in this theorem, that it carries the malt into the stage of over-germination at a quicker rate than would be the case if the germination were conducted in the dark.

In the course of my malting experiments I have also tried to inquire into the behaviour of peroxidases and catalases. The quantitative aspect of this question has not yet been cleared up satisfactorily. At any rate, however, it is certain that barley contains both peroxidases and real catalases. The peroxidases have their principal seat in the periphery of the corn and in the germ, but do not occur in the endosperm itself. The quantity of peroxidases increases in the course of germination, but falls off during kiln-drying. The amount of catalases, on the other hand, does not increase while the barley is germinating; but it declines during the kiln-drying. The rootlets are rich in peroxidases, whilst the leaflets are altogether free from these enzymes.

Kiln-drying (**8**, 362—376).

With regard to the influence of the kiln-drying factors it is to be noticed at the outset that we are here dealing with quite minute effects and changes; indeed, they are so exceedingly inconsiderable that one might feel tempted to assert that the agencies coming into play do not manifest themselves at all.

TABLE IX.

eri- ital ies	Value α = gr. of nitrogen in primary material	Process: Kiln-drying: temperature	hours	with — per cent of water in the malt	Air supplied per hour	Per 10,000 corns: Increase in: dry matter — gr.	extract — gr.	Acid ≙ cc. of normal NaOH	(1) Proteid dissolution	(2) Albumin conversion: Found	(2) Albumin conversion: Calculated	(3) Peptic action	(4) Tryptic action	According to Tables — in original paper
						Numerical value of the process expressed in gr. of nitrogen								
A	0.21	51	4	8.0	Excess	7.6	4.5	1.6	÷0.18	÷0.16	0.03	÷0.16	÷0.20	XL and LXXIII
		60				3.8	2.4	2.4	÷0.14	÷0.01	0.07	÷0.10	÷0.17	
		69				1.7	÷0.7	1.8	÷0.10	0.02	0.11	÷0.07	÷0.11	
		79				4.4	2.2	4.6	÷0.06	—	—	—	—	
		91				1.5	÷2.3	3.9	÷0.03	—	—	—	—	
		100				6.8	÷1.1	4.2	÷0.29	÷0.11	÷0.08	÷0.01	0	
		110				÷4.0	÷7.9	2.8	÷0.39	÷0.18	÷0.18	÷0.29	÷0.16	
B	0.22	60		7.2		÷1.3	2.9	0.7	0.01	0.23	0.21	÷0.01	0.01	XLII and LXXIV
		80				1.3	÷1.3	1.6	÷0.04	0.18	0.18	0.04	0	
		93				0.8	÷2.5	2.6	—	—	—	—	—	
		105				5.0	÷4.4	3.9	÷0.28	÷0.06	÷0.06	÷0.20	0.14	
A	0.22	79	3	7.2	Excess	0.5	÷0.2	÷0.8	0.15	0.21	0.37	0.19	0.18	XLIV and LXXV
		82	5			4.2	1.8	0.6	÷0.06	0.13	0.16	0	÷0.02	
		88	8			7.3	3.5	1.8	÷0.08	0.14	0.14	0.02	÷0.04	
B	0.07	80	1	7.6	0	1.9	0.1	0.1	0.03	0.02	0.10	0.02	0.01	XLVI and LXXVI
			2			2.0	÷1.5	0.2	÷0.02	÷0.05	0.05	÷0.01	÷0.01	
			3			3.0	÷0.7	0.2	÷0.09	÷0.12	÷0.02	÷0.08	÷0.06	
			4			1.8	÷1.9	0.1	÷0.09	÷0.02	÷0.02	÷0.10	÷0.04	
	0.12	80	4	5.4	Excess	÷0.6	÷3.0	÷1.2	÷0.01	0.07	0.11	0.04	0	XLIX and LXXVII
				7.3		÷0.7	÷0.1	0.2	÷0.09	÷0.03	0.03	÷0.04	÷0.12	
				18.4		1.5	0.3	0.7	0.06	0.12	0.18	0.16	0.06	
	0.07	63	3	7.6	0.5 l.	1.6	÷0.5	÷0.3	÷0.01	0.02	0.06	÷0.03	0	LI and LXXVIII
					1.5 l.	÷2.2	÷3.4	÷0.6	÷0.02	—	—	—	—	
					10.0 l.	2.7	0.7	÷0.3	0.05	—	—	—	—	
					Excess	1.0	÷0.2	÷0.6	0.08	0.15	0.15	0.02	÷0.04	
Analytical errors — about						2.1	2.6	3.3	0.12					

All figures without signs are positive.

In pointing out this circumstance, I wish to make a kind of reservation with regard to the way in which I interpret my experimental results. These, as also the agencies referred to, are set forth in Table IX.

If the results are confronted with the analytical errors, it will be seen that different malts are affected somewhat differently by kiln-drying.

The figures relating to dry matter now and then exhibit considerable fluctuations, which are most of them positive, that is, show an increase of dry matter. A considerable negative fluctuation is not met with till the temperature 110° is reached. This goes to prove that no loss of dry matter (oxidation loss) is suffered on the kiln, at any rate not at temperatures below 110°. In this connection we also find that the amount of extract does not decline noticeably till the temperature in the kiln has exceeded 100°. This decline is probably due to an incipient formation of anhydrids or to a caramelisation of certain sugars, but certainly not to a diminution of the power of action of carbohydrate-splitting enzymes.

The figures relating to the acidity are also seen to fluctuate very slightly, generally in such a way that the acidity must be said to increase with temperature in the kiln, at least up to 100°. If this temperature is exceeded, the acidity rather appears to diminish — probably by reason of volatile acids passing away.

A glance at the proteïd transformation processes will show that a greater or less amount of the soluble albumins contained in the malt is almost always coagulated out, process (1) having a negative sign. The coagulation, however, does not take place to any appreciable extent until the temperature of the malt has reached 90°, and it does not appear to be furthered by moisture (see my memoir p. 373). It will also be noticed that both the peptic and the tryptic actions are slightly weakened by kiln-drying; the depression, however, scarcely becomes noticeable till the temperature of the malt has reached 100° (in the case of the tryptic action perhaps a little earlier).

We have seen before that, as soon as germination has been from some reason or other driven more or less out of its normal course, the consequence will almost invariably be an incomplete albumin conversion. The malt will then contain larger or smaller quantities of albumin II, a proteïd which is so very dangerous

to the keeping powers of the beer. Hence it will be understood, why the principal object of my kiln-drying experiments was to clear up the manner in which the various kiln-drying factors act upon albumin II, or in other terms, how they act on a malt in which the albumin conversion process has not been terminated in the course of germination itself. Since albumin conversion has been shown to be closed when we have reached the state of equilibrium expressed by the equation: Process (2) = process (1) + a, it will be an easy matter to throw light upon the question with the help af Table IX, and the more so because the value calculated for Process (2) from this equation and confronted in the table with the value found for the same process enables us to compare the experimental result with the theoretical conclusion. This comparison shows that, after the termination of kiln-drying, the albumin conversion process is at an end in the experiment series I A at 100° and 110°, in I B throughout, in II A after 5 and 8 hours' kiln-drying, in II B after 4 hours, and in IV with excess of air. In the other samples, albumin conversion has not come to a close in the course of kiln-drying.

Thus, we see that albumin II can be removed in kiln-drying from a malt the albumin conversion of which has not been completed in germination. It cannot, however, be ascertained whether albumin II is converted into albumin I or — what seems to be more probable — into an insoluble form. The transformation begins at a temperature of about 60° and increases with temperature and with time, the velocity of transformation increasing with the degree of desiccation of the malt, as also with the amount of air supplied during the drying process But it cannot be brought to an end with equal facility in all malts.

Storage of Malt (**8**, 377—381).

From the annexed Table X it will be seen that the chemical composition of malt undergoes only very slight modifications in the course of the storing period, at least as far as proteïd transformation is concerned.

The figures denoting dry matter warrant the conclusion that — allowance being made for analytical errors — no noticeable loss is suffered by oxidation (respiration) in the course of storage, whereas the amount of extract — and, consequently, also the yield in extract — increases somewhat. This result may probably

TABLE X.

According to Tables LIII and LXXIX in original paper.

Process						(1)	(2)	(3)	(4)	
		Per 10,000 corns								
		Increase in				Numerical value of process expressed in gr. of nitrogen				
Malt stored during — days	Water increased, per cent	dry matter gr.	extract gr.	Acid ∞ cc. of normal NaOH	soluble ash	Proteïd dissolution	Albumin conversion	Peptic action	Tryptic action	Change in extract yield
44	4.07	0	0.8	1.3	0	÷0.09	—	—	—	0.24
79	4.10	÷1.3	1.2	1.1	0.05	÷0.04	÷0.02	0	÷0.08	0.69
115	6.40	2.5	4.7	2.3	0.14	—	—	—	—	0.87
174	7.95	2.7	7.7	4.3	—	0.01	÷0.06	0.05	÷0.03	1.75
223	7.33	0	4.0	5.3	0.15	—	—	—	—	1.24
333	5.09	÷2.0	4.8	8.6	0.55	0.04	0.04	0.08	÷0.06	1.97
Analytical errors — about		2.1	2.6	3.3	0.14	0.12				0.6

be due to the circumstance that the acidity, which is also augmented, tends to enhance the power of some (perhaps all) enzymes.

Not only the acidity, but also the proportion of soluble mineral matters becomes greater as the storage progresses. These two facts are probably attributable to one and the same cause, namely an increase in the amount of primary phosphates.

The peptic action appears to be a little intensified, but tryptic action a little diminished as the storage time goes on.

Finally, the rather inconsiderable variations undergone by the numerical values found seem to indicate that the processes of proteïd dissolution and albumin conversion are impaired in a wet, but furthered in a dry period of storage.

Transformation of Proteïd Matters during Mashing, Boiling of Wort and Fermentation.[1])

All the experiments dealt with in this section were made on malt and wort containing greater or less quantities of the injurious proteïd designated as Albumin II. This implies that

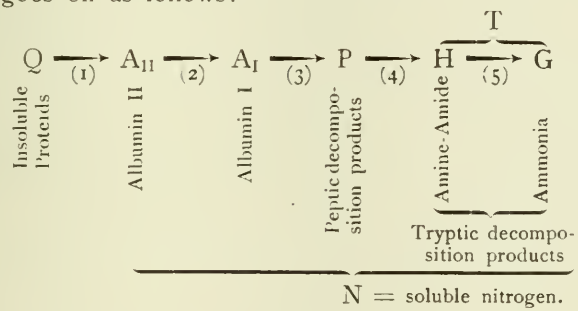
[1]) Compt. rend. des travaux du Laborat. de Carlsberg **9**, 237 (1913).

the albumin conversion in the malt experimented upon had not been brought to a close in the process of germination, my object being to inquire into the behaviour of that particular proteïd in the successive brewing processes (mashing, wort boiling and fermentation).

In most of the investigations I have, besides the aforementioned determinations, effected measurements of the proportion of nitrogen titrable by formol (peptid-bound nitrogen) and of the concentration of hydrogen ions. These measurements were made by the methods devised by S. P. L. Sørensen.[1])

Mashing.

In the process of mashing the proteïd matters contained in the malt undergo the same series of transformations as in malting, setting aside Process (6) — consumption of nitrogen in rootlet-formation. Accordingly, proteïd transformation in the course of mashing goes on as follows: —



The numerical values of the various transformation processes can be calculated from the following two sets of equations, in which the unmarked letters denote the amount of nitrogen corresponding to each group of substances in the initial material, while the marked fellow letters express the parallel values in the experimental material.

Process

(1) = $Q \div Q'$ or $N' \div N$

(2) = (1) + $A_{II} \div A_{II}'$ » $(N' \div N) + (A_{II} \div A_{II}')$.

(3) = (2) + $A_I \div A_I'$ » $(P' + T') \div (P + T)$.

(4) = (3) + $P \div P'$ » $T' \div T$.

(5) = $G' \div G$ » $G' \div G$.

[1]) Compt. rend. des travaux du Laborat. de Carlsberg **7**, 1 (1907), and **8**, 67 (1909).

Before dealing with the question as to the influence exerted by the various mashing factors upon proteïd transformation, it will be as well to examine whether the most intense action occurs in the process of germination or in mashing.

This question can be answered by the aid of the figures recorded in Table XI (**9**, 280—291).

An inspection of the three columns A, B and C will show that the actions which are decisive for the values Extract, Soluble Mineral Constituents and Acidity are represented only by comparatively small or even immeasurable values during the processes of steeping and germination, whereas they assert themselves particularly in the mashing process. From the other series of figures it is evident that the divers proteïd transformations come to pass chiefly in the process of germination. As regards the peptic and tryptic actions, it is only in those cases in which albumin conversion has not been completed in the process of germination that they show almost the same intensity in the process of mashing as in germination. The liberation of ammonia, which is doubtless the last link of the chain of proteïd decomposition brought about by enzymic action, occurs only in germination, but never in the course of mashing. This fact lends some countenance to the notion that the most far-going decomposition of the proteïd molecule takes place in the process of germination. Since, however, the numerical value of ammonia liberation is very small, there is also the possibility that certain rather loosely bound amino groups are immideately separated from the proteïd molecule in the form of ammonia.

Peptid splitting occurs during germination and in that part of the mashing process which extends from 1^0 to 45^0. It is not possible, however, to discover any constant relation between this transformation and any of the other proteïd transformations.

In order to give a clearer idea of the progress of proteïd transformation during steeping, germination and mashing, some of the figures recorded in Table XI are represented graphically in Curve Table 5.

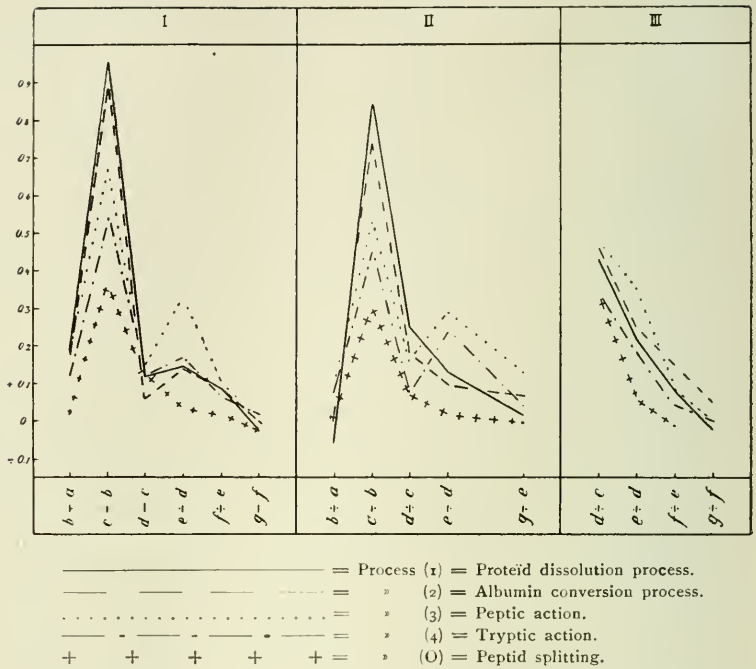
Both the greatest quantitative and perhaps also the most thorough-going qualitative decomposition of the proteïd molecule takes place in the process of germination, where the vital forces are acting, whereas the most important effects in regard to the decomposition and transformation of carbohydrates and mineral

According to Tables XXII and XXIII of original paper.

Experiment No.	Process: Transformation during		A	B	C	(1)	(2)	(3)	(4)	(5)	O	pH· displaced in acid direction	pH· in the malt	Value α expressed in gr. of nitrogen	% of total nitrogen in barley dry matter
			Per 10,000 corns												
			Increase in			Numerical value of process expressed in gr. of nitrogen									
			extract gr.	soluble ash gr.	Acid ∾ cc. of normal NaOH	Proteïd dissolution	Albumin conversion	Peptic action	Tryptic action	Ammonia separation	Peptid splitting				
I	b ÷ a	steeping	2.39	0.23	9.1	0.19	0.18	0.19	0.12	÷ 0.01	0.02	0.1			
	c ÷ b	germination	47.85	÷ 1.15	÷ 1.3	0.96	0.89	0.67	0.54	0.04	0.36	÷ 0.1			
	d ÷ c	mashing from 1° to 45°	18.60	1.46	6.0	0.12	0.06	0.15	0.12	0	0.13	0	6.1	0.14	1.75
	e ÷ d	mashing from 45° to 70°	179.41	0.62	6.0	0.15	0.14	0.33	0.17	0	0.04	0			
	f ÷ e	mashing 1 hour at 70°	7.25	÷ 0.36	1.5	0.09	0.09	0.11	0.07	0	0.02	0			
	g ÷ f	boiling	0.32	÷ 0.05	0	÷ 0.02	0	0	0.02	0	÷ 0.02	0			
II	b ÷ a	steeping	÷ 11.43	—	÷ 1.0	÷ 0.06	0.02	0.10	0.08	÷ 0.01	0.01	0.1			
	c ÷ b	germination	49.63	÷ 0.66	1.6	0.85	0.74	0.53	0.45	0.02	0.30	÷ 0.1			
	d ÷ c	mashing from 1° to 45°	22.71	1.70	12.0	0.25	0.18	0.16	0.08	0	0.07	0	6.1	0.05	1.50
	e ÷ d	mashing from 45° to 70°	178.67	0.86	4.5	0.13	0.10	0.29	0.24	0	0.02	0			
	g ÷ e	mashing 1 hour at 70° + boiling	7.94	÷ 0.02	0	0.02	0.07	0.13	0.04	0	0	0			
III	d ÷ c	mashing from 1° to 45°	22.36	1.18	10.2	0.43	0.46	0.48	0.34	0.01	0.34	0			
	e ÷ d	mashing from 45° to 70°	180.70	0.73	7.2	0.22	0.25	0.35	0.18	0	0.06	0.1	6.2	—	—
	f ÷ e	mashing 1 hour at 70°	9.71	0.07	0	0.08	—	0.08	0.04	0.01	÷ 0.01	0			
	g ÷ f	boiling	1.01	÷ 0.01	0	÷ 0.02	0.05	0	0	0	—	0			
IV α	c ÷ b	germination	—	0.20	6.4	1.45	1.57	1.23	0.93	—	—	—	—		
	g ÷ c	mashing	—	÷ 0.48	÷ 0.4	0.19	0.22	0.44	0.20	—	—	—	—		
IV β	Germinated in 0— 4 days		—	0.12	÷ 0.4	1.04	1.17	1.01	0.74	—	—	—	—	0.15	1.74
	Germinated in 4— 7 days		3.3	÷ 0.40	4.2	0.54	0.56	0.56	0.34	—	—	—	—		
	Germinated in 7—10 days		÷ 10.8		2.2	0.06	0.06	0.10	0.05	—	—	—	—		
	Analytical errors — about		2.6	0.14	3.3	0.07						0.1			

All figures without signs are positive.

Curve Table 5.



constituents occur in the process of mashing, without the direct agency of the vital powers.

The proteïd transformation during germination increases very nearly in proportion to the duration of the latter, up to the moment when the normal transformation is finished.

The greatest effect in point of the conversion of carbohydrates during mashing occurs at a few degrees below 70°. But in regard to the conversion of mineral matters and acid-formation[1]) the optimum lies between 1° and 45°, generally not many degrees below 45°. It is to be remarked, however, that both the peptic and the tryptic action may also increase noticeably in the course of the mashing process (the optimum lying between 45° and 70°); this is the case when proteïd transformation has not been completed before the close of germination (short malt). If proteïd transformation is completed in the process of germination (long

[1]) The change occurring in these two values is probably due to one and the same cause, namely the conversion of organic phosphides and tertiary phosphates into secondary and primary phosphates.

malt), the action of proteolytic enzymes will hardly be furthered by the mashing process to any appreciable extent [1]).

It is also evident that in those malts (I, II and III) in which albumin conversion has not been brought to a close in the process of germination, the fault cannot be rectified or removed in mashing.

The speed with which proteïd transformation in germinating barley is accomplished is by no means in an inverse ratio to the total nitrogen amount of dry matter. Consequently, the fitness of a barley for malting purposes depends not only on its vitality or behaviour in the process of steeping, but also on the length of time it requires to bring the proteïd transformation to an end during germination.

The measurements of the numerical values of the different proteïd transformations by my precipitation method, and of the extent of peptid splitting through a formol titration, act as supplements to one another, but cannot replace one another. The proteïd transformation processes mark the proteolysis down to the formation of the most complex amine-amide compounds (polypeptides?), whilst the peptid-splitting particularly marks the decomposition of the most complex into the less complex amine-amide compounds. The peptid splitting gives no information as to the qualitative aspect of proteolysis (**9**, 291—295).

If we are now to inquire into the influence exerted by the different mashing factors upon the processes of transformation, it must be constantly borne in mind that the comparisons are based upon a normal mashing carried out by the exact method of Proportionality, as follows: —

50 gr. of finely ground meal of malt of known amount of water are mixed up (mashed) with 200 cubic centimetres of distilled water at a temperature of 45°, which is maintained for twenty minutes and then raised in the course of twenty-five minutes to 70°. After the mixture has been kept at this temperature for one hour, it is slightly boiled over an open gas-light (reckoned as one minute's boiling), when it is directly cooled down in ice water to 15°, completed with distilled water to exactly 450 gr., and filtered.

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[1]) On account of the circumstance that there is not much material left for the enzymes to act upon.

Experiment No.	Process: Mashing — Factor	Process: Mashing — changed to		Extract yield of malt dry matter increased by — %	Increase in: soluble ash gr.	Increase in: Acid 2 cc. of normal NaOH	(1) Proteid dissolution	(2) Albumin conversion	(3) Peptic action	(4) Tryptic action	(5) Ammonia separation	0 Peptid splitting	Increase in: Peroxidase	Increase in: Catalase	pH displaced in acid direction	According to Table — of original memoir	Standard factor
							Numerical value of process increased by — gr. of nitrogen										
I	Fineness of malt meal	Coarse Meal		÷ 0.54	0	0	÷ 0.3	0.5	0.5	0	÷ 0.2	—	—	—	—	XXV	Fine Meal
		Groat		÷ 1.17	0.05	0	÷ 0.8	0	÷ 0.4	÷ 0.4	÷ 0.2	—	—	—	—		
II	Concentration of mash	100 cc. of water		÷ 0.31	0.02	0	0.8	0.8	0.8	0.4	0.1	—	—	—	—	XXVI	200 cc. of water
		300 cc. of water		0.53	0.14	0	÷ 0.8	÷ 1.1	÷ 0.8	÷ 1.6	0.1	—	—	—	—		
		350 cc. of water		0.10	0.08	0	÷ 1.2	÷ 2.6	÷ 1.5	÷ 2.0	0	—	—	—	—		
III	Mashing Temperature	The samples mashed one hour at temperature	10°	1.38	0.20	0.9	2.1	2.0	0.6	0.3	—	0.7	÷ 0.2	1.2	0	XXVII	1 hour at 1°
			20°	3.71	—	2.2	4.6	3.5	1.7	1.1	0.2	2.1	2.4	1.0	0		
			32°	6.51	0.64	3.1	8.1	8.4	6.3	4.6	—	4.2	0.6	1.1	0		
			38°	8.20	—	3.9	9.8	9.0	8.3	6.3	0.2	5.3	÷ 0.6	1.3	0		
			45°	14.06	0.72	4.8	11.2	11.2	10.8	7.7	—	5.3	÷ 0.4	1.0	0		
			50°	31.62	0.72	4.4	10.5	10.1	10.8	6.6	0.2	3.5	—	—	0		
			55°	44.66	0.73	3.9	9.8	10.1	11.5	6.3	—	2.8	÷ 0.4	0.3	0		
			61°	58.68	0.64	3.5	7.0	—	9.4	5.9	0	2.1	—	—	0		
			65°	58.89	0.65	3.1	6.0	5.7	8.5	5.3	—	1.4	÷ 0.2	÷ 0.2	0		
			71°	57.53	0.43	0.9	1.7	2.5	5.5	2.0	0	0.3	—	—	0		
			76°	57.21	—	0.4	0.7	1.7	5.2	1.4	—	0	÷ 1.2	0	0		
			81°	56 48	0.40	÷ 0.4	÷ 2.8	÷ 0.7	2.8	÷ 0.4	0	÷ 1.4	—	—	0		
IV	Duration of Initial Mashing	0 minute		÷ 0.63	÷ 0.05	÷ 0.9	÷ 1.5	÷ 1.9	÷ 1.5	÷ 1.1	÷ 0.2	—	—	—	—	XXVIII	20 minutes
		30 minutes		0	0.04	0	0.4	0.5	0.5	0.4	0	—	—	—	—		
		60 minutes		0.21	0.03	0.4	0.7	1.2	0.4	0.7	0	—	—	—	—		
V	Duration of Boiling	0 minute		÷ 0.20	0	0	÷ 0.3	÷ 0.3	÷ 0.7	÷ 0.3	0	—	—	—	—	XXIX	1 minute
		10 minutes		÷ 0.31	÷ 0.04	0	÷ 0.7	÷ 1.5	0	0.4	0	—	—	—	—		
		20 minutes		÷ 0.20	÷ 0.01	0	÷ 1.5	÷ 2.7	÷ 0.8	0	0.2	—	—	—	—		
VI	Elutriation of Husks	elutriated — times	1	4.80	0.05	0.4	2.3	2.9	2.6	1.3	0.2	—	—	—	—	XXX	0
			2	5.95	0.12	0.6	—	—	—	—	0	—	—	—	—		
			3	8.81	—	1.0	2.9	3.1	1.8	1.4	0.2	—	—	—	—		
Analytical errors — about				0.2	0.06	0.4	0.4—0.7								0.1		

All figures without signs are positive.

The following mashing factors will be made the object of our considerations in connection and comparison with the results obtained by this standard method: — Degree of Fineness of malt meal, Concentration of mash, mashing Temperature, Duration of preparatory mashing, Time of Boiling of mash, Elutriation of husks, and Salt effect.

The experimental results regarding these factors (excepting the last) are summarised in Table XII, which also states the ways in which the experimental factors were varied.

Experiment I shows that the yield of extract diminishes noticeably and the amount of soluble nitrogenous matter very little, as the degree of fineness of malt meal becomes lower. The ratio of the amount of extract and that of soluble nitrogen, however, do not undergo any appreciable change (**9**, 296).

The influence exerted on the different transformations by the Concentraton of mash (**9**, 297—300), that is, the ratio of the quantity of malt meal to the amount of water with which it was mixed, is shown by Experiment II in the same Table XII. The changes observed, which are by no means immaterial, are mainly due to the fact that the formation of extract — that is the transformation of carbohydrates — increases as the concentration of mash is diminished, the maximum of extract formation lying at the concentration 1 part of malt meal to 6 parts of water, whereas the proteïd transformation decreases as the concentration decreases, the maximum lying at 1 part of meal to 2 parts of water, if not at a still greater concentration.

By varying the concentration of the mash we may from the same malt prepare different worts in which the ratio of total extract to total nitrogen is changed, — in the present instance as follows: —

1 part of malt meal mashed with — parts of water	100 parts of extract contain — parts of total nitrogen
2	0.819
4	0.799
6	0.777
7	0.774

It is further seen that proteïd transformation is also influenced qualitatively by the factor under consideration, in that the proteïd dissolution process (1) decreases in about the same proportion

as the concentration of mash, whereas the peptic action (3), tryptic action (4), and especially the albumin conversion (2) decrease somewhat more. Accordingly, we cannot take it for granted that we may without any reservation consider this mashing factor as capable of regulating the relation between extractive matters and total nitrogen, unless albumin conversion has been brought to an end in the course of germination.

The faculty of clarification of the wort and the time in which it runs off decrease a little as the concentration decreases.

As regards the mashing Temperature (**9**, 300—305), its great influence on the quantitative proteid changes was established by previous inquirers. The figures set forth in our Table XII, series III, show that it exerts a marked influence also upon the qualitative progress of proteolysis, the cause of which must be sought in the fact that the peptase has its optimum at about 55°, whilst that of the tryptase and the proteïd transformation generally lies in the neighbourhood of 45°. Moreover, the tryptase has an optimum point which is as well-marked as that of the peptase (not an optimum zone).

Neither the liberation of ammonia nor the concentration of hydrogen ions is influenced by the mashing temperature.

The action of peroxidases increases from 10° to 20°, but decreases as soon as the temperature exceeds 20°; at 38° the action has almost ceased. — The catalases exercise their maximum action at 10°, and only begin to be weakened at temperatures above 45°. — In the finished brewery wort both peroxidases and catalases have been entirely destroyed.

The right duration of Initial Mashing (**9**, 305—308) exerts a favourable influence on the various transformations taking place during the principal mashing (see Table XII, series IV), and more particularly on the albumin conversion process. Nevertheless, however, it will be difficult, if not impossible, to attain a perfectly completed albumin conversion if working with a malt where the proteïd transformation has not been brought to a close in the process of germination. The liberation of ammonia remains unaffected by this factor.

The duration of initial mashing is of great consequence and may be utilised as a regulator, but only within certain limits. If prolonged, at 45°, beyond ½—1 hour, it will even be apt to work mischief.

About the direct Boiling of the mash (**9**, 309—310) there is not much good to be said. It appears from Table XII, series V, that nearly all the chemical changes brought about by the boiling go in negative directions. This holds good particularly of the processes (1) and (2), according to which albumins coagulate and the quantity of albumin II becomes greater with the time of boiling. It cannot be decided with certainty whether this increase is due to a partial conversion of albumin I into albumin II, as suggested in my memoir, — or to a further dissolution of the edestin salts existing in the malt. As the clarifying faculty of the wort diminishes in the progress of boiling, any misuse of this factor must be warned against.

The Washing out of the Husks with distilled water (Elutriation) (**9**, 310—312) does not exert any material influence upon the composition of the extract. —

The concentration of hydrogen ions is not appreciably affected by any of the above-mentioned factors.

The Salt Effect.

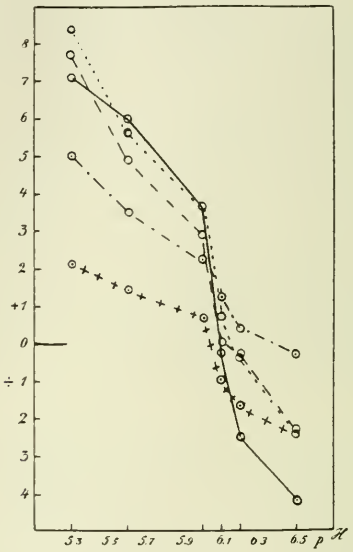
The way in which the mashing processes are acted upon by this factor was examined by the aid of various acids, bases and salts, the concentration of which varied from 0.06 to 0.005 gr. equivalents in 1000 cc. of mashing water. The numerical results are recorded in Tables XXXII and XXXIII in my memoir (**9**, 312—329).

Equivalent quantities of different anions exert the same effect upon all the proteïd transformation processes, whereas a slight difference is perceptible in their power of reaction towards the carbohydrates. In the latter case the sulphate ion acts a little more vigorously than the chlorine ion, whether the effect be positive or negative. Equivalent quantities of different kations exert very nearly the same effect upon all the transformation processes. The anions exert a favourable, the kations a restraining influence, and equivalent quantities of the different anions and different kations neutralise each other in regard to their action.

The various transformations are influenced in the process of mashing in three different ways by the salts existing in the mashing water, namely: by the change of hydrogen ion concentration and acidity, and by the amount of salt. The greatest influence on the course of the transformations is exerted by the

hydrogen ion concentration, and the least influence by the salt amount. If the hydrogen ion concentration, the acidity or the salt concentration increases or is augmented (as a matter of course, not beyond a certain point), the transformation processes are pro-

Curve Table 6.



= Process (1) = Proteïd dissolution process.
= » (2) = Albumin conversion process.
= » (3) = Peptic action.
= » (4) = Tryptic action.
= » O = Peptid splitting.

moted to a greater or less degree, whereas they are checked if the numerical values of these three factors are diminished one by one or simultaneously.

The salt amount itself (the direct salt effect) has a slightly favourable influence on the transformation of carbohydrates and albumin II, and perhaps also on the peptic action. The optimum of salt amount could not be ascertained.

The transformations of carbohydrates and albumin II are changed in the same direction as the acidity. The quantitative effect is, however, not very great.

The hydrogen ion concentration is apt to influence the transformation of carbohydrates and all the proteïd transformation processes, excepting ammonia liberation. The influence rises or

falls as the hydrogen ion concentration rises or falls; quantitatively it is pretty considerable, and it is always greater in regard to the peptic than to the tryptic action. The maximum effect requires a hydrogen ion concentration superior to $10^{\div 5.8}$, and the minimum effect a concentration inferior to $10^{\div 6.8}$.

A graphic representation, as given in Curve Table 6, shows that nearly all the proteïd transformation processes are greatly influenced by a modification of the hydrogen ion concentration answering to a change of the exponent from 6.2 to 6.0. Thus, they are very sensitive to changes in the hydrogen ion concentration, especially if the latter is comprised between the values $10^{\div 6.2}$ and $10^{\div 6}$.

The change in hydrogen ion concentration is mainly brought about by the phosphates contained in the malt, which shows a hydrogen ion concentration corresponding to the hydrogen ion exponent 6.1 or to a phosphate mixture of the composition: $3\ Na_2HPO_4 + 17\ NaH_2PO_4$. The salts present in the mashing water may be supposed to act upon this mixture according to the following equations:

(I) $3\ Na_2HPO_4 + 17\ NaH_2PO_4 + Na_2SO_4 =$
$3\ Na_2HPO_4 + 17\ NaH_2PO_4 + Na_2SO_4$

(II) $3\ Na_2HPO_4 + 17\ NaH_2PO_4 + Na_2CO_3 =$
$5\ Na_2HPO_4 + 15\ NaH_2PO_4 + H_2O + \underbrace{CO_2}_{\text{volatile}}$

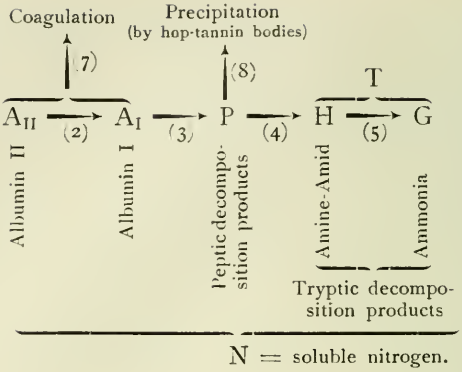
(III) $3\ Na_2HPO_4 + 17\ NaH_2PO_4 + CaSO_4 =$
$2\ Na_2HPO_4 + 17\ NaH_2PO_4 + Na_2SO_4 + \underbrace{CaHPO_4}_{\text{indissoluble}}$

(IV) $3\ Na_2HPO_4 + 17\ NaH_2PO_4 + CaCO_3 =$
$4\ Na_2HPO_4 + 15\ NaH_2PO_4 + \underbrace{CaHPO_4}_{\text{indissoluble}} + H_2O + \underbrace{CO_2}_{\text{volatile}}.$

According to equation (I) the hydrogen ion concentration does not change, according to equation (II) it decreases, by equation (III) it increases, and according to (IV) it decreases, though less than by (II).

Wort Boiling.

As might have been supposed, the proteïd transformation taking place in the course of wort- and hop-boiling may be summarised by the following scheme (**9**, 329—363):



As the numerical values of Processes (7) and (8) cannot be determined separately, but only in total, the action of the hops upon the proteïd substances can be followed with certainty only as far as the tryptic action and ammonia liberation are concerned. The values of the different processes can be calculated by means of the following equations:

Boiling without hops.

Process

(7) = N' ÷ N = Coagulation (as a negative value).
(3) = (P' + T') ÷ (P + T) = Peptic action.
(4) = T' ÷ T = Tryptic action.
(5) = G' ÷ G = Ammonia liberation.
(2) can be calculated only if (7) = 0, in which case we have (2) = A_{II} ÷ A_{II}'.

Boiling with hops.

Process

(7 + 8) = N' ÷ N = Coagulation + Precipitation.
(7 + 3) = (A_I + A_{II}) ÷ (A_I' + A_{II}') = Peptic action + Coagulation.
(8 + 3) = (P' + T') ÷ (P + T) = Peptic action ÷ Precipitation.
(4) = T' ÷ T = Tryptic action.
(5) = G' ÷ G = Ammonia liberation.

The factors examined are: Duration of Boiling (332—340), Proportion of Hops (340—345), Boiling Temperature (345—351), Wort Concentration (351—355), Oxygen Tension (355—359) and Sort of Hops (359—363). The quantitative effects exerted by these factors on the various transformation processes are generally very inconsiderable, and may be summarised as follows: —

The extractive matters are but very slightly affected by the wort- and hop-boiling. The changes brought about are quantitatively dependent on the extent to which the coagulation and precipitation processes have advanced.

The figures expressing acidity and hydrogen ion concentration increase with the time of boiling, quantity of hops and temperature of boiling, but are not affected by wort concentration and oxygen tension, and only slightly by the sort of hops.

The coagulation process (7) increases with the time of boiling (maximum about 4 hours) and with the proportion of hops. It reaches its maximum in the vicinity of 78^0. Oxygen also appears to act favourably on this process.

The precipitation process (8) attains its maximum after four hours' boiling; if the boiling is continued, the process becomes reciprocal. It increases with the quantity of hops and appears to be favoured by the oxygen of the air.

The albumin conversion process (2) shows a very low numerical value. It increases with the duration of boiling and reaches its maximum after four hours at a pressure of one atmosphere. Albumin II does not, as a rule, appear to coagulate in the wort- and hop-boiling, unless a very large quantity of hops is used; and even then only a small proportion of albumin II is thrown down.

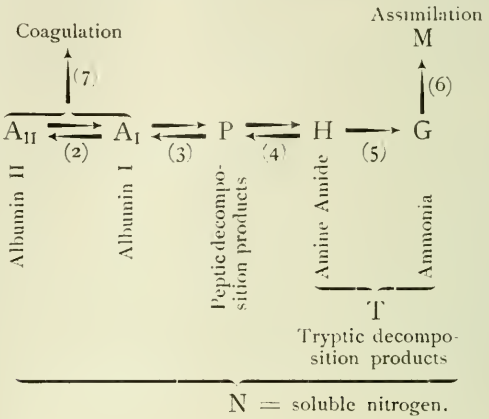
The changes undergone by peptic (3) and tryptic (4) cleavage are in the main dependent upon the processes of coagulation and precipitation: The greater the numerical values of these, the less are the changes observed in peptic and tryptic decomposition. In this respect worts prepared with different malts are seen to diverge considerably; normally we have a satisfactory coagulation and precipitation, and consequently weak peptic and tryptic actions.

The liberation of ammonia (5) is found to increase with the time and temperature of boiling, but not much. An increased quantity of hops also tends to augment the amount of ammonia in the wort; but this augmentation is due to the hops themselves.

As for the influence exerted by different sorts of hops, it deserves notice that some sorts promote coagulation and precipitation more than others, whilst in regard to the other changes no difference is perceptible.

Fermentation (**9**, 363—387).

It may be considered as an established fact that the transformation undergone by the nitrogenous substances of the wort (nutrient liquid) in consequence of the action of living micro-organisms may be expressed by the following scheme (363—367):



As before, the unmarked letters denote the values found for the different substance-groups contained in the initial material (the nutrient liquid before fermentation), while the marked letters represent the corresponding values in the fermented liquid after the latter has been filtered from the developed yeast. From these figures we can calculate the numerical values of the different transformation processes. As, however, the action of some organisms follows the upper, and that of other organisms the lower series of arrows, it must be supposed that the processes always go in both directions It will be the simplest plan to always calculate the values of the processes from the lower arrows, because this side of the transformation generally predominates. We then arrive at the following equations:

Process

(6) = M = Assimilation process.

(7) = (N ÷ N') ÷ M = Coagulation process.

(3) = (P + T) ÷ (P' ÷ T') ÷ M = Condensation of peptic decomposition products.

(4) = T ÷ T' ÷ M = Condensation of amine-amide compounds.

(5) = G' ÷ G + M = Ammonia liberation.

(2) cannot be calculated.

If we find the two proteolytic condensation processes to have negative signs, this will simply mean that the proteolytic decompositions have the upper hand of the proteolytic condensations, and conversely if the signs are positive.

The fermentation experiments were made with the following five microorganisms: Saccharomyces Carlsbergensis, Saccharomyces Pastorianus I, Saccharomyces apiculatus, Torula B (Brettanomyces) and Monilia candida. The annexed Table XIII gives the results obtained in regard to Saccharomyces Carlsbergensis (those relating to the other organisms are recorded in Tables L and LI of my memoir (**9**, 368 and 372).

The five species examined were found to differ widely in respect of their vital working powers. The greatest working power is possessed by Saccharomyces Pastorianus; next follow Sac. Carlsberg., Monil. cand., Sac. apic., the least power being exhibited by Torula B. The oxygen of the air favours the vegetative propagation of the aërobian organisms, but impairs the vital working power of the cell. The vitality seems to be weakened or even destroyed if hydrogen ion concentration exceeds $10^{\div 4.2}$.

The hydrogen ion concentration increases in the course of the primary fermentation, but diminishes somewhat in a sufficiently prolonged secondary fermentation. — It is the same with the acidity. The different organisms, however, are far from having the same tendency to form acids or acid compounds, this tendency being seen to increase in the following order: Sac. Carlsberg. (*nil*), Monil. cand., Torula B, Sac. Pastor., Sac. apic. This order is, however, changed if air be supplied during the course of fermentation; Torula B then forms an enormous amount of acid.

A small proportion of the mineral constituents of the nutrient liquid is, of course, consumed in the new-formation of cells. —

A summary view of the different processes of proteïd transformation is given in Curve Table 7.

It will be an easy matter to distinguish the five organisms from one another by means of the physiologico-chemical changes brought out in this Curve Table, namely by observing, with respect to each organism, the mutual situation of the three curves relating to Assimilation, Ammonia liberation and Peptid splitting, due regard being also paid to the direction (positive or negative)

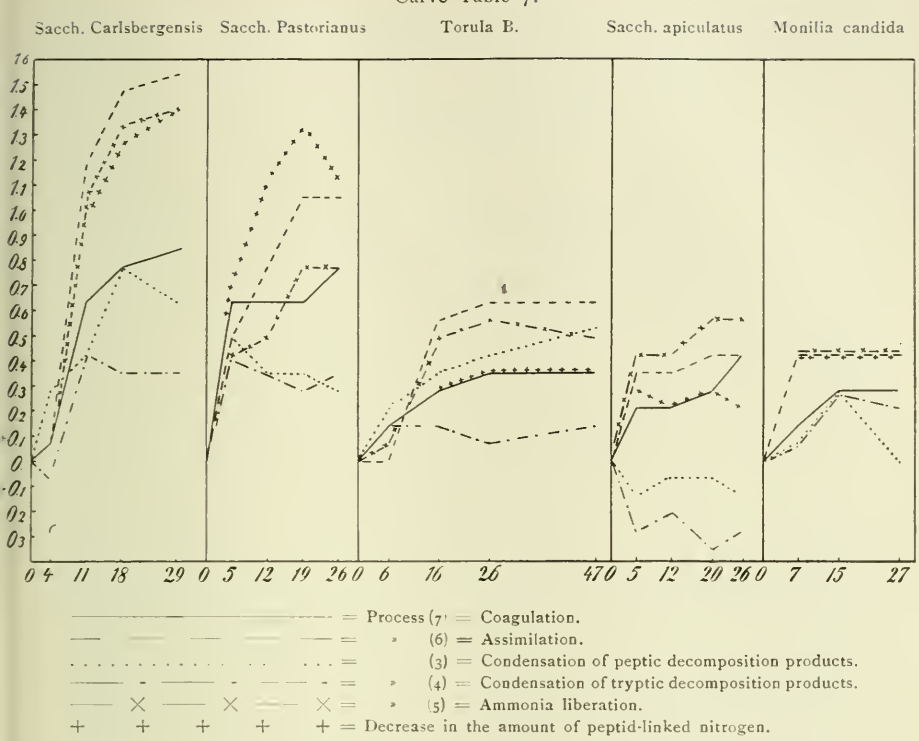
TABLE XIII.

Column marks			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
The results calculated			per 10,000 cc. of experimental liquid														
Found for process			Consumed			Increase in acidity (∞ cc. of normal Na OH)	pH displaced in acid direction	(7)	(6)	(3)	(4)	(5)	O÷O'	Gr. of extract consumed per gr. of assimilated nitrogen	% of total nitrogen of the standard wort consumed in		
Standard wort fermented at 15° to 17°								Numerical value of process expressed in gr. of nitrogen									
with		— days	gr. of extract	% of extract contained in standard wort	gr. of ash			Coagulation	Assimilation	Peptic Condensation	Tryptic Condensation	Ammonia liberation	Decrease in peptid bindings		Coagulation	Assimilation	Sum
Saccharomyces Carlsbergensis	without air supply	4	19	1.5	0.5	0	0	0.07	0.07	0.28	÷ 0.07	0.07	0.07	271	0.7	0.7	1.4
		11	650	51.1	0.9	(?) 15	0.2	0.63	1.19	0.42	0.42	1.05	0.98	546	6.0	11.3	17.3
		18	811	63.8	1.4	0	0.4	0.77	1.47	0.77	0.35	1.33	1.26	552	7.3	14.0	21.3
		29	826	64.9	1.9	0	0 4	0.84	1.54	0.63	0.35	1.40	1.40	536	8.0	14.6	22.6
	with air supply	4	7	0.6	÷ 0.3	0	0	0.07	0.07	0 28	÷ 0.07	0.07	0.07	100	0.7	0.7	1.4
		11	747	58.8	1.2	25	0.2	0.98	1.82	0.56	0.49	1.47	1.26	411	9.3	17.3	26.6
		18	811	63.8	1.8	20	0.8	0.98	1.82	0.77	0.35	1.54	1.68	445	9.3	17.3	26.6
		29	811	64.6	2.0	30	0.8	0.91	2.03	0.42	0.28	1.75	1.75	404	8.7	19 3	28.0
Anal. errors — about			2.0		0.6	4.0	0.1	0.07					0.11				

All figures without signs are positive.

(?) The determination appears to be incorrect.

Curve Table 7.



of the two proteolytic transformations. The organisms considered act very differently — both quantitatively and qualitatively — upon the nitrogenous matters contained in the nutrient liquid, which means that different organisms are able to split different bindings in the molecules of nitrogenous organic compounds.

The greatest transformations within the group of nitrogenous matters occur during primary fermentation, and the oxygen of the air tends to promote these transformations, though not always in the same measure as it promotes vegetative propagation.

The vegetative propagation is possible even though the nutrient liquid does not contain any such nitrogenous matters as can be utilised by the microorganism in the service of nutrition. But in the latter case the new-formed cells contain a protoplasm which is practically devoid of nitrogen. These cells are incapable of inducing alcoholic fermentation.

May 1914.

JOHS. SCHMIDT:

INVESTIGATIONS ON HOPS

(*Humulus lupulus*, L.).

IV. A METHOD FOR QUANTITATIVE DETERMINATION OF RESINS IN HOPS.

BY

Ö. WINGE and J. P. H. JENSEN.

(Received for publication, June 22nd 1914.)

IN the series of investigations on hops which under the direction of Dr. Johs. Schmidt have occupied the Physiological Department of the Carlsberg Laboratory it appeared desirable to find a purely numerical expression for the brewing value of different samples of hops. This valuation of the hops has hitherto been made, and still is made, almost exclusively by estimation of purely external factors, as the colour of the hops, the amount of lupulin, aroma and the like, and consequently can be little more than an individual judgment, very open to chance causes; the judgment is uncertain and thus not suited to form the basis for a comparison of the different kinds of hops or the crops of different years of the same hops, which in the present investigations would be of importance.

An objective estimate of the value of hops must naturally have its basis in the determination by chemical means of the hops components of value in the brewing processes and as the hops investigations of the Carlsberg Laboratory necessitated making a numerous series of investigations within a relatively brief period of time, the task was to find an analytical method, which in a rapid and trustworthy manner and without asking too much of the experimental skill of the tester could give a true numerical expression of the valuable constituents of the hops. Various suggested methods are certainly to hand, but the considerable differences which appear on comparing the results of different analysts show already, that the question has not been solved

satisfactorily; it deserves to be taken up again for further treatment and a closer study of the methods and their basis strengthens this opinion.

The solution of the task in question had therefore to be sought in a closer study of what is to be understood by the valuable components of the hops and in working out a simple method for the quantitative determination of these.

1. The valuable components of the hops.

Of the components that come into consideration, when it is a question of determining the value of a hops sample, weight has been laid on the tannins, the aromatic oils and the resins. A large quantity of all these three is considered as a rule to be advantageous, and in the case of the aromatic oils and the resins further the quality is considered to be of importance.

Tannins. As is known, the importance of the tannins is, that on passing into the wort they help to cause the precipitation of the albumens. It appears from previous investigations, that the hops always contains sufficient quantities to cause the intended precipitation of the albumen in the wort. Chapman (1907 and 1909) has indicated a method for the determination of the tannin in hops and we have used it with good results in several of our investigations. The proceedure is as follows: 10 gm. hops (Chapman uses whole hops, but as in our resin determinations we have used crushed hops) are placed in a glass retort with a mark at 508 cc.; 400 cc. boiling water are added and the whole boiled on a water bath for 2 hours. The mixture is then cooled to about 15^0 and filled up to 508 cc. The liquid is filtered and 50 cc. of the filtrate is evaporated on the water bath down to 15 cc., cooled, 50 cc. saturated cinkonin sulphate solution added and the whole left to stand for 2 hours. The liquid is now filtered through a weighed Gooch crucible with asbestos and the precipitate washed with a 0.5 per cent. cinkonin sulphate solution, and the crucible is also washed with this before drying and weighing. The crucible with the precipitate is dried at 100^0 to constant weight. The weight of the precipitate, multiplied by 0.55, gives the quantity of tannic acid in 1 gm. hops. As a rule the quantity of tannic acid varies between 2 and 5 per cent. calculated on the dried stuff of the hops. It has no great interest, however, to determine the exact percentage

of tannic acid, as this can easily be added to the wort in another way, if desired, for example by addition of other tannin-rich materials, hops leaves or the like cheap products.

Hops oil. The stuff in the hops which probably is considered to be the chief factor in producing the bouquet of the beer, is an aromatic oil, which can be isolated from the hops by distillation (Chapman 1898). Clemens Horst (1912) is of opinion, that during the boiling of the wort the hops oil is removed and that the aromatic value of the hops is therefore of subordinate importance. Lintner (1911) likewise states, that a great part of the hops aroma is lost into the air during boiling, but maintains nevertheless, that practice shows that the aroma of the beer depends a great deal on the fineness of the hops used. Hayduck (1888) shows, that the aromatic oil by exposure to the influence of the sunlight and air changes over into the form of resin. The resin produced in this way is insoluble in petroleum ether, but soluble in most other dissolving media and is almost certainly identical with the γ resin mentioned later.

A careful and very valuable investigation of the chemical and physical properties of the hops oil, its quantitative determination and variation in different kinds of hops has been published in May 1914 by Frank Rabak.

Resins. Investigations on the resins of hops have been in progress since the middle of the nineteenth century. We need not cite here the many works dealing with the purely chemical conditions of the resins, but may refer in this connection to the papers of M. Issleib, H. and A. Bungener, C. I. Lintner and G. Barth.

Hayduck (1887 and 1888) was the first to carry out experiments of practical value, in so far as he closely studied the solubility and taste of the resins. He finds — and his results have later been confirmed by others — that the hops contains three different resins, α and β resins, the so-called soft resins, and γ resin, the hard resin. α resin is a thick, brown, very bitter stuff, soluble in petroleum ether, ethyl alcohol, ethyl ether and other similar dissolving media, but only to a small extent soluble in water. The substance has a disinfecting action as regards bacteria, especially lactic acid bacteria. β resin is similar to α resin in most properties; it is soluble in petroleum ether, slightly soluble in water, with a bitter taste, but is somewhat less poison-

nous towards the organisms mentioned. γ resin is a solid substance without bitter taste and with little or no power to hinder the development and activity of the lactic acid bacteria. Like the two others γ resin is soluble in ethyl ether, ethyl and methyl alcohol, carbonic disulphide etc., but insoluble in petroleum ether. Hayduck found also, that the soft resins on oxidation change partly into the γ form. He found all the resins present in the scum of the fermenting vats as also in the finished beer. He was able to separate the bitter, soft resins from the tasteless, hard resin owing to their different solubilities in petroleum ether. The soft resins could only be separated from each other by their conduct towards lead salts; when a suitable quantity of lead acetate is added to a mixture of the dissolved substances, the α resin is separated out by the formation of an insoluble lead salt, whilst the β resin formed no lead salt. Thus for Hayduck, where it was a question of a practical estimate of the hops as a brewing material, it was only necessary to determine the quantities of the α and β resins, whereas the γ resin owing to its lack of taste could be left quite out of consideration.

Whilst Hayduck (1887) stated in his first report on the hops resins, that both the hard γ resin and the soft α and β resins were bitter, in his later work (1888) he reports that this conclusion was due to an error, since the β resin is so closely connected with the γ resin, that it cannot be completely separated by means of petroleum ether. It is only after repeated boiling with water, that a γ resin not bitter can be obtained. Hayduck sums up his views on these points as follows (l. c. p. 941): »Nur diese beiden Harze ertheilen dem Bier den gewünschten bitteren Geschmack und besitzen die wichtige Eigenschaft, Spaltpilzgährungen zu hemmen. Das γ Harz besitzt keine der beiden Eigenschaften. Es ist im Bier enthalten, muss aber als wertloser Bestandtheil angesehen werden«.

Almost all later authors seem to agree with Hayduck, that the hard resin is valueless, and this view has characterized the different analytical methods, which all lay weight on the separation of the soft and hard resins. Our experiments, however, have shown us, that the resins which the analytical methods hitherto used have deducted from the total quantity of resins as valueless must in reality be regarded as valuable.

Among the experiments which have convinced us of this we have the following. The resins obtained by vaporising an ethyl ether extract of lupulin glands are freed from wax by dissolving in methyl alcohol and again separated from this by distillation in a vacuum. With frequent shaking they are then mixed with plenty of petroleum ether for 5 hours, after which the precipitate, a strongly yellow powder, is obtained by filtering and washed with petroleum ether until this runs through colourless and gives no turbidity with a solution of lead acetate in methyl alcohol. The powder is now left to stand in petroleum ether for 24 hours, again washed and dried. This yellow powder, which on melting or dissolving in ethyl ether and evaporation of this changes to a hard, brown, amorphous mass, is thus the hard γ resin, as isolated in the quantitative analyses.

The soft resins, freed from the hard resin, are prepared by diluting the above-mentioned filtrate from the hard resin with petroleum ether. This causes the precipitation of the small quantities of hard resins, which owing to the great concentration of soft resins in the petroleum ether had been dissolved in these (see p. 128). The precipitate is filtered off and the solution vaporised to dryness in vacuum. Then the soft resins obtained in this way are dissolved in methyl alcohol and added to a saturated solution of lead acetate in methyl alcohol. (As much lead acetate is used, as corresponds to 25 per cent. of the quantity of resin, taking the formula of the lead salt to be $C_{20}H_{30}O_5Pb$ (Siller, 1909)). The precipitate is filtered off after standing 2 hours and to the filtrate is added drop by drop a 1 per cent. lead acetate solution until the liquid which stood above the precipitate gives a lead reaction with sodium sulphide. The solution is filtered after 2 hours and vaporised in vacuum; the residue after vaporisation is dried in vacuum for 24 hours at 35° then dissolved in petroleum ether and the solution is filtered from a small quantity of lead salt still present. The filtrate is evaporated and the residue again dried in vacuum. The substance obtained in this way may be considered as fairly pure β resin.

The above-mentioned lead salt was repeatedly washed with petroleum ether, until this ran through colourless, then allowed to settle in the petroleum ether and placed with an equal volume of sulphuric acid (1:3) in a separating funnel and shaken well

for 5 minutes; after the liquid had separated, the petroleum ether solution was removed by evaporation — and the residue dried in vacuum for 24 hours. The partially crystalline mass thus obtained may be regarded as pure α resin.

A series of taste experiments was now made with the α, β and γ resins obtained in this way, samples of the same wort being boiled with respectively 0.3 gm. α, β or γ resin to each liter, which approximately corresponds to the quantity of hops used in the breweries. Various unbiassed persons took part in judging the taste and the result of the experiments was, that after boiling for 1 hour there was but a slight bitter taste in the wort boiled with γ resin, the β resin had given the wort a somewhat more bitter taste and that boiled with the α resin was already very bitter. The wort boiled without resin could not be filtered clear, whereas all three samples to which resin had been added were clear after filtering. — After 2 hours' boiling the sample with γ resin was very distinctly bitter, yet less bitter than the sample with β resin, which again was less bitter than the sample with α resin, — and after 4 hours' boiling the difference became still less; yet a difference could be detected between the wort samples in the order α, β, γ. For the boiling time generally used in brewing, the relative bitterness of the wort samples boiled with α, β and γ resin respectively might be indicated approximately by the proportion $\alpha : \beta : \gamma = 10 : 7 : 4$.

When the γ resin is boiled repeatedly with water, it remains constantly bitter. On shaking the aqueous solution with ethyl ether this becomes yellow, whilst the water loses its taste; but if the aqueous solution is shaken up with petroleum ether, this does not become coloured and the water retains its bitter taste. The bitter substance is thus insoluble in petroleum ether. The resin remaining after repeated boiling becomes on cooling hard, glass-like and brittle, — and it can be understood, why Hayduck thought he could produce a non-bitter substance in this way, for the bitterness of the hard resin cannot be ascertained directly; it is only in the dissolved form (in weak alcohol, for example) that the bitter taste is detected. On boiling the γ resin for several hours in many lots of water we have always obtained a residue, which gave an apparently just as bitter solution when dissolved in alcohol, although this residue was in a form and condition widely different from the original hops resin.

From these experiments, which have been varied in different ways, we may conclude that the γ resin is bitter, and as it likewise has a clearing effect on the wort, it must also be considered to have a certain brewing value. It would thus be wrong not to take it into account in the quantitative determination of the bitter substances in hops.

2. Analytical methods.

When we began our investigations in 1911, we turned naturally to the method proposed by Briant and Meacham (1897) which is based on the view, that petroleum ether only dissolves the soft α and β resins, but not the hard γ resin, the last of which, as mentioned, is generally considered to have no bitter taste and on the whole no value for the brewing process. In Briant and Meacham's method whole hops are extracted in the Soxhlet apparatus with petroleum ether to determine the soft resins and thereafter with ethyl ether, which dissolves the remaining γ resin and the determination is made by weighing the dried extracts. Previous to the weighing Hantke (1898) has introduced a treatment of the petroleum ether extract with methyl alcohol, which dissolves the soft resins, but not the plant wax dissolved by petroleum ether, the presence of which naturally leaves an error in the determination. The method gave us results which agreed well with each other but they showed a surprisingly large quantity of the hard resin by comparison with the more recents results of Fischer (1911), though they agree with the observations of others who have worked closely with Briant and Meacham's method, as will be seen from the following comparison (Table I).

TABLE I.

Analyst	Hops	Percentage contained in hops of		
		soft resins	hard resin	
Remy 1897	—	10,26 9,09	8,17 8,29	average of 8 analyses
Hantke 1899	Californian New York lupulin glands	9,14 8,05 58,12	6,04 7,06 35,85	
Biró 1909	Hungarian hops	12,48	6,04	average of a series of analyses
Carlsberg Laboratory 1911	Semsch Rak.	6,9	3,4	average of 5 analyses

It seemed reasonable to suppose, that the high values for the γ resin given by the method arose from the very incomplete extraction by the petroleum ether of the soft resins from the hops, and experiments soon showed, that of the dried ethyl ether extract, which should thus represent the hard γ resin, a considerable portion was dissolved in the petroleum ether.

This observation led us to try the method, which according to Briant and Meacham was suggested by Delbrück (and which has been used later by Tartar and Bradley in 1912): to extract the hops in the Soxhlet apparatus with ethyl ether for 24 hours, distil off the ether, dry the extract, which on weighing gives the total quantity of resins, decoct the extract with cold petroleum ether, filter and distil off the solution and then by weighing the dried extract determine the quantity of soft α and β resins. — Briant mentions in his investigations, that Delbrück's suggestion is not suitable in practice, since the extract obtained by extraction with ethyl ether cannot again be dissolved in ethyl ether and thus must have undergone an essential change on drying. This condition has never been observed in our experiments. The evaporated, vacuum-dried extract always gave a quite clear solution on dissolving again in ethyl ether.

The agreement between the different experiments made according to Delbrück's method was however, as appears from Table II, not very satisfactory, which we believe to be due mainly to the fact, that the ether was inclined to remain stationary in the Soxhlets apparatus, as it only flowed down from the one side into the siphon and thus was not always in operation. Consequently, the ether sometimes became warm in one, sometimes in another apparatus and thus extracted different quantities of the resin and chlorophyll present.

TABLE II.

Sample	a	b	c	d	e	f	g	h
% total resins . . .	13,56	13,37	13,30	12,60	12,56	12,69	12,75	13,35
% soft resins	11,49	11,84	11,47	11,20	11,09	11,06	11,38	11,74
% hard resins . . .	2,07	1,53	1,83	1,40	1,47	1,63	1,37	1,61

For some time we sought in vain for a means to get over these shortcomings and at once took up a suggestion put forward by Dr. Johs. Schmidt to try whether it was possible to extract the resins directly with cold ether without using the Soxhlet apparatus. — Our experiments in this direction were made as follows. A number of samples of about 6 gm. of the hops, referred to in Table II, after being dried in vacuum at 40° for 24 hours were placed with ethyl ether in Erlenmeyer retorts of 300 cc. The different samples stood for different times; the liquid was filtered off and distilled, then the extract was dried in vacuum and weighed. The extracted hops however were again placed with ether in the same manner to see what quantity of resin could still be extracted. All the extracts were then treated with petroleum ether to determine the quantity of soft resins. The results are brought together in Table III.

TABLE III.

Sample	a	b	c	d	e	f	g	h	i	k
	1st extraction									
Days	1	1	2	2	3	3	4	4	5	5
% total resins. . .	12,10	11,82	12,78	12,58	12,52	12,20	12,20	13,17	13,15	12,33
% soft resins . . .	10,56	10,30	11,75	11,13	10,83	11,19	11,48	12,16	11,81	—
	2nd extraction									
Days	1	5	2	4	1	3	4	2	5	1
% total resins. . .	1,25	1,67	1,13	1,24	0,84	1,02	0,98	0,77	1,12	0,93
% soft resins . . .	1,54	1,59	0,62	1,03	0,62	—	0,71	0,38	1,09	1,00
% total resins sum of totals . .	13,35	13,49	13,81	13,82	13,36	13,22	13,18	13,94	14,27	13,26

It will be seen, that on extracting the hops in two portions cold ether over a varying number of days, just as high values were obtained as by extraction in the Soxhlet apparatus (see Table II). It will be seen also, that a longer period of standing with the ether, both in the first and the second extraction, is of no importance, the values not becoming any higher. We were obliged to conclude, that the second extraction was in the main

only a washing out of the already dissolved resin and we then tried with another sort of hops to obtain results from extractions of respectively 2 days + 2 hours and 2 days + 1 day. Table IV shows clearly that the longer treatment does not give higher values than the shorter.

TABLE IV.

Period of extraction		2 days + 2 hours	2 days + 1 days
% total resins.	I. . .	16,91	16,60
	II. . .	16,36	16,71
	III. . .	17,14	16,69
Average.		16,80	16,66

For a long time thereafter we used this method in practice to determine resins in hops grown in the experimental garden of the Laboratory. For each determination we used 6 gm. hops, treated with 300 cc. ether for 2 days; the liquid was filtered off and the hops again treated for 2 hours with a similar quantity of ether, after which the two ether solutions were distilled in the same, weighed retort. After drying in vacuum at 40° for 24 hours and weighing the total quantity of resin was determined, and lastly the hard and soft resins were separated by treatment with petroleum ether for 4 hours. This petroleum ether extract was filtered, distilled, dried and also weighed. — The determination takes 5 days and might therefore be thought unpractical. But since the analyses can be left to themselves most of the time, it is quite easy, in making a series of analyses, to arrange the whole in a system, so that a large number can be carried out each day.

Along with these analytical determinations an investigation was also made into the dissolving power of petroleum ether towards the hops resins.

The ordinary, trade petroleum ether of low boiling point, which is generally used for resin determinations in hops, is stated to have boiling points from 30°--50°. To investigate the extracting power of the different components several fractional distillations of the trade product were made, 1.5 l. being fractioned by heating on the electric water-bath and the receivers

cooled on ice. The results are shown in Table V. The four distillates II, III, IV and V were used for the extraction of hops (»Grøn Humle«). Owing to the low boiling points the extraction which lasted 4 days, was made at a temperature of 9°. The results as also the density of the distillates after completion of the extraction are also shown in Table V.

TABLE V.

No. of distillate	Volume[1] (cc.)	Boiling point	Density (15°)[2]	% resin	Density after the extraction
I.	35	12—18°	0,625[3]	—	—
II.	350	17—23°	0,622	5,43	0,626
III.	350	23—27°	0,628	4,39	0,630
IV.	350	27—37°	0,636	3,81	0,636
V.	350	37—52°	0,651	3,72	0,651
VI.	35	52—58°	0,679	—	—

It appears from this, that petroleum ether with low density and low boiling point, used directly on the hops, more readily extracts this than one with a high boiling point. — On the other hand where it is a case of extracting the soft resins with petroleum ether from a vaporised ether extract, it seems, that the high boiling components of the petroleum ether extract a larger quantity of resin than the low boiling; our experiments in this regard gave the following results.

TABLE VI.

Hops sample »Grøn«	% total resins in the hops (ethyl ether extr.)	Density of fractions used in the extraction (15°)	Approx. boiling point	% soft resins in the hops (extr. with petroleum ether of the dried ethyl ether extract)	% hard resins in the hops (difference)
A.	14,94	II 0,626	23°	11,66	3,28
B.	14,95	III 0,630	28°	12,23	2,72
C.	15,00	IV 0,636	32°	12,85	2,15
D.	15,26	V 0,651	45°	13,14	2,12

[1]) During distillation altogether 30 cc. were lost by evaporation to the air.

[2]) Measured with Westphal's weight.

[3]) The determination, which was carried out at 12°, is uncertain, as currents were already present in the liquid at this temperature owing to incipient boiling.

The explanation of this probably is, that the more volatile fractions of the petroleum ether, which have a smaller friction coefficient, more readily penetrate the hops than the higher boiling fractions, but that the latter are able to dissolve larger quantities of the hard resins and thus get the upper hand in the case of the extraction of the pure substances in readily accessible form.

The temperature has considerable influence on the completeness of the extraction. Two series of distillates of petroleum ether were used in a 4 days' extraction of the same kind of hops at respectively 9° and 15°. Although the fractions in the two series have not exactly the same densities, it is clear from Table VII nevertheless, that the least dense (0,626) at 9° extracts a smaller quantity of resin than the most dense (0,652) at 15°.

TABLE VII.

Temperature of extraction	9°		15°		
Distillate series	Density (15°)	% resin	Distillate series	Density (15°)	% resin
a	0,626	5,43	a^1	0,633	6,31
b	0,630	4,39	b^1	0,637	6,08
c	0,636	3,81	c^1	0,641	5,97
d	0,657	3,72	d^1	0,652	5,92

The density of a quantity of petroleum ether increases appreciably during use, as it is essentially the most volatile components that are lost; the consequence is, that the liquid used gradually obtains a higher boiling point and higher density. Even if we add fresh petroleum ether in case of need to the quantity of liquid used, owing to the loss from evaporation, yet we do not obtain a liquid with approximately constant density. In an experiment where the stock of fresh petroleum ether had the density 0,633 (bp. ca. 32°) its density during use was 0,643 (bp. ca. 39°).

It must be evident from this already, that such a heterogeneous mixture as petroleum ether is not suited for the quantitative determination of the soft hops resins. Feuerstein (1911) has come to the same result, but thinks it possible nevertheless to

retain the petroleum ether as means of extraction, as he »adulterates« the petroleum ether by addition of benzine, so that the boiling point of the mixture is maintained for example at 45 °, and Adler (1912) states — as also several others — that petroleum ether of the same boiling point should always be used for the determination of the soft resins. Results apparently in agreement can be obtained by these means, but they are nevertheless quite misleading, since the γ resin, which is insoluble in petroleum ether, is soluble in a solution of the soft α and β resins in petroleum ether, and all the more the greater the concentration of the soft resins is — a condition, that can be clearly seen when a concentrated solution of »soft resins« in petroleum ether is diluted with petroleum ether, this causing the precipitation of the resin.

Thus the petroleum ether method does not give the separation of the soft from the hard resins that is desired, and since our experiments mentioned on p. 119—122 on the valuable components of the hops led us to the view, that such a separation has no essential importance, but that chief weight should be laid on the determination of the total resins in the hops, we directed our efforts to working out a reliable method which should be easy in practice.

Instead of treating the ethyl ether extract with petroleum ether, as mentioned on p. 125, we followed Hantke in using a methyl alcohol treatment to eliminate the error due to the wax present in the extract and in this way, as shown in Table VIII, obtained extremely consistent results, so long as it was a case of analysing unpressed hops from the hops culture of the Laboratory. It is further seen from Table VIII, that ethyl ether extracts just as great quantities by weight of moist and dry hops.

TABLE VIII.

% water contents	% resins			Average
	Sample a	b	c	
7,99	15,55	15,34	15,68	15,52
3,14	15,69	15,62	15,69	15,67
0,00	15,68	15,42	15,46	15,52

In the case of the pressed, trade goods however, the method proved to be impracticable, for the greatly compressed condition of the inflorescences caused the resin to be extracted imperfectly. We were obliged therefore, to introduce the method proposed earlier and used by Neumann (1910), namely, to crush the hops by means of a meat mincing machine. — Owing to the finely divided form in which the hops was thus exposed to the ethyl ether, much larger quantities of chlorophyll, fatty substances of the fruits etc. are extracted than in the ether treatment of whole hops inflorescences, and the weighing results will consequently be too high. It was thus preferable to determine the resins by titration instead of by weighing, as proposed by C. J. Lintner (1898).

Lintner boils 10 gm. hops for 8 hours with 300 cc. petroleum ether in a retort with return condenser; after filling up to 505 cc. with petroleum ether 100 cc. of the filtered solution are titrated with 0,1 n. alcoholic potassium hydroxyde solution; the results are calculated on the assumption, that the monobasic lupulin acid ($C_{25}H_{36}O_4$) as β resin composes by far the greater part of the hops resins, that the remainder of these are monobasic acids and have almost the same molecular weight, and that 4 cc. n. potassium hydroxyde solution thus corresponds to 1 gm. resin.

To ascertain, if we could find in practice some factor that could be used in calculating the titration analyses, we adopted the following proceedure.

20 samples of different kinds of hops (whole hops) were extracted in the ordinary manner at the room temperature with ether in Erlenmeyer retorts, the extract was filtered, distilled and dried in a vacuum apparatus from which the atmospheric air was driven out by means of carbonic acid. After drying for 24 hours at 35 ° the resin was treated with pure methyl alcohol, the solution filtered and distilled in vacuum. The resins were dried again in the same manner as above mentioned, cooled in sulphuric acid exsiccators and carefully weighed. The mass in the retorts must therefore be considered a pure mixture of hops resins. After weighing the 20 extracts were again dissolved each in 150 cc. neutral ethyl ether, and the titration was made with 0,04 n. potassium hydroxyde in 93 % alcohol, using a 1 % phe-

nolphtalein solution in 93 % alcohol. The results from this experiment are given in the accompanying Table IX.

TABLE IX.

No. of sample	Quantity of resins in gm.	cc. 0,04 n. KOH used	To 1 cc. 0,04 n. KOH corresponds gm. resin
1	0,894	56,2	0,0159
2	0,318	19,7	0,0161
3	0,832	50,6	0,0164
4	0,873	53,9	0,0162
5	0,773	48,0	0,0161
6	0,627	38,8	0,0162
7	0,571	36,4	0,0157
8	0,380	23,9	0,0159
9	0,730	44,4	0,0164
10	0,519	32,2	0,0161
11	0,848	52,1	0,0163
12	0,424	26,4	0,0161
13	0,793	49,6	0,0160
14	0,713	44,9	0,0159
15	0,740	47,0	0,0157
16	0.920	56,2	0,0164
17	0,184	11,3	0,0163
18	0,212	13,1	0,0162
19	0,317	19,8	0,0160
20	0,241	15,2	0,0159
		Average . . .	0,01609

The figures show, that the proportion between the quantity of potassium hydroxyde used and the quantity of resin is constant within the experimental errors and that to 1 cc. 0,04 n. potassium hydroxyde solution corresponds 0,01609 gm. resin, thus to 1 cc. n. potassium hydroxyde solution corresponds 0,402 gm. resin.

Thus, in a practical way we have found a factor which is in good agreement with Lintner's statement, that the β resin is present in greatest quantity, has the molecular number 400 and is a monobasic acid.

Since we have found, however, that the bitterness of the resins can be represented by the proportions $\alpha : 10 = \beta : 7 = \gamma : 4$, it might seem quite unpermissible to take the percentage of total resins as expression for the degree of bitterness of the hops. It will be seen from the following, however, that this is permissible when the determination is made by titration.

In our tasting experiments, namely, it always proved that the total resins and the β resin were almost equally bitter, and the following calculation shows this to be in agreement with the bitter factors given above. If, for example, we have a hops with 16 per cent. total resins, experience shows that it will have on an average 6 per cent. α, 8 per cent. β and 2 per cent. γ. Multiplying each of these percentages with the respective bitter factors of the resins and adding the three results, we obtain a number which represents the »total bitterness« of the hops: $6 \times 10 + 8 \times 7 + 2 \times 4 = 124$. On dividing this sum with the percentage of total resins we obtain the bitterness factor of these: $124 : 16 = 7,7$, which thus is almost equal to the bitterness factor of the β resin 7.

On determining the titration factors for α, β and γ resin we have found that

to 1 cc. n. KOH corresponds 0,32 gm. α resin
0,40 gm. β resin
ca. 0,6 gm. γ resin.

If we make the same calculation with these as with the bitterness factors, we find the »total acidity« to be $6 \times 0,32 + 8 \times 0,4 + 2 \times 0,6 = 6.32$ and from this the acidity factor or titration factor of the total resins $6,32 : 16 = 0,395 =$ ca. 0,4.

As this agrees with the titration factor found earlier, we can thus say in general, that the total resins occurring in hops act as if they were β resin both with regard to bitterness and titration determination. It may be objected to this, that the different kinds of hops may contain the resins in a proportion differing a good deal from the average composition mentioned here; this however will be of subordinate importance, for a hops which contains much α resin, thus is very bitter, will have a higher percentage quantity, determined from the titration by using the factor 0,4, than hops of average composition. In the same way a hops with a large quantity of γ resin, thus of little bitterness, will give in the titration a low percentage of resins.

We are convinced therefore, that a determination which gives an approximately correct impression for the value of the hops, expressed by its contents of resins, is most easily and best obtained by titration of an ethyl ether extract of the hops. From this standpoint we have worked out the following ether—cold extraction method.

30 gm. hops are pulverised by means of a meat machine. The first 5 gm. are discarded, and the remainder amounting to ca. 15 gm., part remaining in the machine, is carefully mixed. About 5 gm. corresponding to a volume of about 25 cc., are taken out by means of a measuring glass and poured into a 300 cc. Erlenmeyer retort weighed beforehand. The retort is weighed with the hops, then dried in a vacuum for 24 hours at 35°, cooled over sulphuric acid and again weighed; the loss of weight gives the water contents. 150 cc. ethyl ether free of water and alcohol are now added and the mixture left to stand about 1 hour with repeated shaking. The liquid is then filtered into an Erlenmeyer retort and a careful washing with ethyl ether is made by means of a water bottle with fine spray. This washing both of the hops powder and of the filter is of great importance and about 100 cc. ether are used for this purpose. The washing completed the solution is titrated with 0,05 n. potassium hydroxide solution in 93 % alcohol, using 6—8 drops of a 1 % phenolphtalein solution in 93 % alcohol as indicator. The titration is continued until the solution has become so deep red, that no colour change occurs on further addition of potassium hydroxyde. — Since to 1 cc. n. potassium hydroxyde corresponds 0,40 gm. resin, the desired percentage contents of resins, based on the dry weight of the hops, becomes

$$x = \frac{y \cdot 0{,}4 \cdot 100 \cdot n}{z}$$

where y is the number of cc. KOH solution used, n the normal of this solution and z the dry weight of the hops in grammes.

Using 0,05 n. KOH solution,

$$x = \frac{2y}{z}$$

An example of the scheme we use in practice is given in Table X, and the example, which in this case embraces 6 samples, likewise gives a picture of the accuracy of the titration.

TABLE X.

Hops	Golding	—	—	—	—	—
No.	905	—	—	—	—	—
When crushed	19/2 1914	—	—	—	—	—
Retort No.	14	8	27	29	6	58
Retort weight	60,528	52,095	47,693	60,496	58,620	47,812
+ moist hops	65,761	57,667	52,988	66,136	64,067	53,043
+ dry hops	65,561	57,463	52,797	65,932	63,868	52,853
Moist hops	5,233	5,572	5,295	5,640	5,447	5,231
Dry hops	5,033	5,368	5,104	5,436	5,248	5,041
Loss in gm.	0,200	0,204	0.191	0,204	0,199	0,190
% water contents . . .	4,0	3,8	3,7	3,8	3,8	3,6
Retort No.	28	22	23	21	30	9
cc. 0,05 KOH.	31,3	33,3	31,3	32,8	32,0	31,0
% resin	12,4	12,4	12,3	12,1	12,2	12,3

The details indicated in the method, as the duration of the various processes (drying, extraction) and standardisation of the titration liquid etc., have not been taken by chance, but are the result of the thousands of determinations which have been made in the course of time.

Since the resins, as has often been shown, can only bear heating to a small extent, we have set the drying temperature as low as 35°, at which temperature no appreciable change occurs in the resins during the time required for drying. In a series of experiments, one of which is shown in Table XI, we have found, that 24 hours' drying in vacuum at 35° gives much the same loss of weight as on drying to constant weight in vacuum over sulphuric acid at the room temperature. The amount of resin also agrees in the two cases; but if the heating to 35° is continued for several days, the hops loses steadily in weight and the amount of resin is found always less owing to the incipient destruction.

TABLE XI.

Drying in vacuum over sulphuric acid at ordinary temperature

No. of days	4		5		7	
	% H_2O	% resin	% H_2O	% resin	% H_2O	% resin
a	3.3	12,4	4,2	12,5	3,8	12,6
b	3,4	12,4	4,2	12,2	4,0	12,3
c	3,4	12,3	4,1	12,5	4,0	12,5
Average	3,4	12,4	4,2	12,4	3,9	12,5

Drying in vacuum at 35°

No. of days	1		2		3	
	% H_2O	% resin	% H_2O	% resin	% H_2O	% resin
a	4,7	12,9	5,4	12,1	6,1	11,4
b	4,9	12,2	5,7	12,1	6,0	11,0
c	4,7	12,3	5,8	12,2	6,0	11,6
Average	4,8	12,5	5,7	12,1	6,0	11,5

Drying at a higher temperature, e. g. at 105° for 3 hours, as proposed for the determination of water in the carbon tetrachloride method (see p. 141) gives according to our experiments, one of which is shown in Table XII, far to high percentages of water, the hops tissues being broken down. The experiment embraces 10 samples of the same hops, 6 of which were dried 1 hour at 105°, the other 6 dried 24 hours in vacuum at 35°.

TABLE XII.

	Dried 1 hour at 105° C.					
	I	II	III	IV	V	VI
% water	9,7	10,0	8,8	9,0	8,7	9,2
% resin	5,1	5,0	5,2	5,0	5,5	5,2
	Dried 24 hours in vacuum at 35°					
	VII	VIII	IX	X	XI	XII
% water	5,7	5,6	5,7	5,7	5,8	5,7
% resin	6,7	6,8	6,7	6,6	6,8	6,7

In using a mincing machine, as Neumann (l. c.) originally proposed for cutting up the hops, it might be feared, that the lupulin would stick fast in the machine to such an extent, that true average samples of the crushed hops could not be obtained. The hops certainly loses some resin in a clean machine, but a counterbalance soon sets in, so that if the machine is simply blown through between each analysis, it is only necessary to discard the first 5 gms. of each hops sample in order to be certain, that the succeeding hops mass is uniform and has the correct proportion of lupulin. Nor does the quantity of water in the crushed hops differ from that in the whole hops, when we use a mincing machine turned by hand and take care, that the rate of turning is not so great, that the machine becomes appreciably warm. — Of the experiments which have convinced us of this, the following may be mentioned.

50 gms. of a hop (Semsch, Rakonitz) were taken and about 5 gms. whole hops picked off; the rest was pounded in the mincing machine and collected in samples of ca. 6 gm. as the cutting up process proceeded. In all 5 samples were taken and these along with the above-mentioned sample of whole hops were placed to dry in vacuum. The results of the moisture determinations are shown in the accompanying Table XIII, where also the result of the subsequent determination of the resin contents is added.

TABLE XIII.

	Whole hops	Crushed hops				
Sample No.		1	2	3	4	5
% water . . .	6,7	6,5	6,7	6,7	6,6	6,6
% resin . . .	15,8	16,3	15,7	15,9	15,7	15,9

The first sample of crushed hops (1) gives here as in our other experiments a percentage of resin, which differs somewhat from the others. The reason for this must be, that on blowing through the mincing machine mainly the dry parts of the leaves are removed, whilst the sticky glands remain and thus cause the first sample to be too rich in resins; as stated above this must therefore always be rejected.

When a large number of analyses have to be made daily,

however, the hand machine is inconvenient and we have therefore adopted a motor crushing machine[1]), which in the main gives just as good results; the amount of water in the crushed hops is certainly somewhat lower, but in general this is of no importance. If it is desired to determine the quantity of water very exactly, the best proceedure is to dry the whole hops in vacuum. Table XIV contains the results of 20 analyses all made on the same hops sample; half of the analyses were made on whole hops, in the other half the hops were cut up in the motor crushing machine according to the rules given in the description of the method. The Table also gives some idea of the influence of the sampling on the accuracy of the results.

TABLE XIV.

Analyses of crushed hops

	I	II	III	IV	V	VI	VII	VIII	IX	X	Aver.
% water	4,9	4,8	4,8	4,9	4,6	4,6	4,4	4,3	4,6	4,7	4,7
% resin	13,9	13,1	14,0	14,1	13,9	14,6	13,9	14,5	13,8	13,7	14,0

Analyses of whole hops

	I	II	III	IV	V	VI	VII	VIII	IX	X	Aver.
% water	5,6	6,0	5,8	5,9	5,9	5,3	6,2	6,6	6,0	5,9	5,8
% resin	14,7	14,4	14,3	14,5	13,9	14,3	14,4	13,9	14,4	14,0	14,3

To determine what influence the duration of the extraction has on its completeness we have made a series of experiments, of which we restrict ourselves to giving some few in Table XV.

TABLE XV.

No. of hours extracted	1/2	1	2	4
% resin	14,1	14,4	14,5	14,6
	14,1	14,7	14,5	14,7
	14,3	14,5	14,3	14,5
Average	14,2	14,5	14,4	14,6

[1]) Supplied by Alexanderwerk with 1/4 H. P. motor.

TABLE XV (fortsat).

No. of hours extracted	1	2	3	4	24	48
% resin	14,7	14,6	14,5	14,4	14,6	14,6
	14,7	14,4	14,2	14,5	14,7	14,7
	14,5	14,4	14,4	14,5	14,6	14,6
	14,5	14,5	14,3	14,4	14,5	14,6
Average	14,6	14,5	14,4	14,5	14,6	14,6

It appears from this, that one hour's extraction is sufficient.

As mentioned above, it is necessary for the extraction to use perfectly pure ether; otherwise, the hops tannic acid, which is insoluble in pure ether but soluble in ether containing water, goes over into the extract. From experiments with hops tannic acid (prepared according to Etti, 1876) we have found, that 0,2 per cent of water in the ether is sufficient for this in 2 hours' extraction to dissolve so much tannic acid, that 100 cc. ether requires over 1 cc. 0,05 n. potassium hydroxyde to give a permanent colour with phenolphtalein. Further, it is just as necessary to have the hops dried before the extraction as it is to use pure ether. This condition however only appears in the titration analysis, as it is a question of very small quantities by weight of hops tannic acid (of p. 128).

Table XVI below contains the results of extraction of the same hops in undried and dried condition.

TABLE XVI.

	Not dried. 4,5 % water contents	Dried in vacuum with sulphuric acid at the room temperat. to constant weight	Dried in vacuum at 35° 24 hours
% resin	12,4	11,4	11,4
	12,7	11,4	11,5
	12,6	11,4	11,4
Average	12,6	11,4	11,4

TABLE XVII.

Samples	A			B			C		
Analysis	a	b	c	a	b	c	a	b	c
Render No. 484									
gm. dry hops . . .	3,998	3,992	4,028	5,548	5,300	5,388	4,684	4,568	4,661
cc. 0,05 n. KOH .	26,3	27,8	26,9	36,3	34,6	34,7	30,4		29,7
% resin	13,2	13,9	13,4	13,1	13,1	12,9	13,0	+	12,7
Average	13,5			13,0			12,9		
Grön No. 1017									
gm. dry hops . . .	3,925	4,283	4,529	5,510	5,166	5,474	4,444	4,246	4,450
cc. 0,05 n. KOH .	26,7	28,1	30,1	34,6	31,8	34,9	29,7	30,0	30,7
% resin	13,6	13,1	13,3	12,6	12,3	12,8	13,4	14,1	13,8
Average	13,3			12,6			13,7		
Mathon No. 760									
gm. dry hops . . .	4,522	4,481	4,794	5,292	5,334	5,131	4,356	4,375	4,061
cc. 0,05 n. hops .	38,9	39,8	41,6	43,9	44,9	42,4	37,8	38,9	35,1
% resin	17,2	17,8	17,4	16,6	16,8	16,5	17,4	17,8	17,3
Average	17,5			16,6			17,5		
Mathon No. 770									
gm. dry hops . . .	5,397	5,176	5,581	5,262	5,280	4,963	3,972	4,147	4,523
cc. 0,05 n. KOH .	40,7	38,3	41,2	41,6	41,5	38,5	31,9	33,0	36,0
% resin	15,1	14,8	14,8	15,8	15,7	15,5	16,1	15,9	15,9
Average	14,9			15,7			15,9		
Brambling No. 646									
gm. dry hops . . .	5,446	4,652	5,447	5,089	5,082	5,240	5,893	5,426	5,552
cc. 0,05 n. KOH .	35,9	29,9	35,5	33,8	34,7	35,5		37,9	37,9
% resin	13,2	12,9	13,0	13,3	13,7	13,5	+	14,0	13,7
Average	13,0			13,5			13,8		
Brambling No. 720									
gm. dry hops . . .	4,269	4,222	4,335	4,947	5,244	5,078	4,394	4,124	4,388
cc. 0,05 n. KOH .	30,6	31,1	31,6	33,7	35,4	33,9	29,3	27,0	28,8
% resin	14,3	14,7	14,6	13,6	13,5	13,4	13,3	13,1	13,1
Average	14,5			13,5			13,2		

Those marked with + were lost during the analysis.

TABLE XVII (cont.).

Samples	A			B			C		
Analysis	a	b	c	a	b	c	b	a	c
Saaz No. 113									
gm. dry hops . . .	4,996	4,916	4,626	6,713	6,400	6,512	4,915	4,714	4,683
cc. 0,05 n. KOH .	28,5	28,5	29,6	37,8	36,4	35,7	27,7	25,6	25,8
% resin	11,4	11,6	11,5	11,3	11,4	11,0	11,3	10,9	11,0
Average	11,5			11,2			11,1		
Saaz No. 422									
gm. dry hops . . .	5,947	6,510	6,001	5,531	6,207	5,779	4,543	4,714	4,289
cc. 0,05 n. KOH .	36,6	38,0	36,5	35,8	39,2	37,2	28,1	27,6	27,1
% resin	12,3	11,7	12,2	12,9	12,6	12,9	12,4	12,8	12,6
Average	12,1			12,8			12,6		
Dauba No. 577									
gm. dry hops . . .	4,937	5,121	5,398	6,510	6,717	6,461	4,467	5,064	4,828
cc. 0,05 n. KOH .	25,8	26,7	28,0	34,0	35,2	32,8		24,9	24,6
% resin	10,5	10,4	10,4	10,4	10,5	10,2	+	9,8	10,2
Average	10,4			10,4			10,0		
Schwetzinger No. 138									
gm. dry hops . . .	5,154	5,329	5,395	5,755	5,782	5,716	6,283	5,942	5,747
cc. 0,05 n. KOH .	39,6	39,1	40,5	45,8	44,9	44,4	49,8	48,2	45,0
% resin	15,4	14,7	15,0	15,9	15,5	15,5	15,9	16,2	15,7
Averag e.	15,0			15,6			15,9		
Oregon Kent No. 100									
gm. dry hops . . .	5,054	5,028	5,308	5,448	5,657	5,516	4,648	4,896	4,610
cc. 0,05 n. KOH .	31,2	29,3	32,2	34,2	36,9	35,3	31,1	32,6	30,3
% resin	12,3	11,7	12,1	12,6	13,0	12,8	13,4	13,3	13,1
Average	12,0			12,8			13,3		
Washington Nr. 428									
gm. dry hops . . .	5,294	5,631	5,506	5,980	6,181	5,992	4,594	4,602	4,788
cc. 0,05 n. KOH .	27,9	30,4	28,9	30,4	32,4	30,1	24,5	24,4	25,7
% resin	10,5	10,8	10,5	10,2	10,5	10,0	10,7	10,6	10,7
Average	10,6			10,2			10,7		

Those marked with + were lost during the analysis.

It appears from this, that even so small a quantity of water in the hops as 4,5 per cent increases the percentage of resin found by over 1 per cent.

Owing to the sometimes greater, sometimes less amount of chlorophyll and the like contained in the ether extracts, it is not possible to determine the point of the colour change during titration by comparison with a standard colour and, as mentioned earlier, the titration has to be continued until further addition of potassium hydroxyde solution does not give a deeper red colour. The fixing of the position of this point is subject to a small variation according to the view of different analysts and the agreement between the analyses of several persons is thus less than the agreement between the results of the single analyst. The accompanying Table XVII shows the results of a series of analyses made by three different persons. The experiment embraces 12 kinds of hops; of each of these each of the three persons took a sample of ca 50 gm., in the Table marked respectively A, B and C, and ground it in the motor crushing machine; from each of the three crushed portions each of the three persons took a sample of ca. 5 gm., in the table marked respectively a, b and c and with these carried out the complete determination, extraction and titration. The result of each single analysis and the average of the three analyses of each sample are given and show, that the variation in the sampling is so preponderating in proportion to the variation within the three analyses, that the disagreement arising from the different estimates of the three persons during the titration and whole accomplishment of the analysis is of subordinate importance.

It is readily understood, that one can counteract a chance variation in the hops material to be analysed by taking and crushing larger samples than those indicated in our method; but as a rule, especially when the hops contains a large quantity of water, the mincing machine will become warm when a large quantity is crushed at one time; further, the quantities used by us have proved to give sufficiently concordant results. For these reasons we have not laid any weight on large samples in our method of analysis.

For the economical working of the method it is necessary to recover the ether and this we have done in the following manner.

After the titration the ether is collected in a large bottle which is half full with saturated salt solution (1 part salt to 3 parts water) and passed through 5 such bottles in the course of 5 days, the bottles being well shaken twice daily, and the liquid thereafter distilled on the electric water bath, the temperature being not allowed to rise above 40° C. The ether solution is then dried for some days with calcium chloride and finally again at least ten days with metallic sodium, then distilled on the electric water bath at 35° C. — Whether this is made with sufficient care can most readily be tested by comparing the results of a couple of hops analyses in which it is used with a couple for the same hops extracted with pure ether.

To give some notion of the cost of our method we may note here the quantities of ethyl ether, alcohol — and sodium and calcium chloride for drying the redistilled ether, — we use for 1000 analyses.

74 l. ethyl ether.
35 l. ethyl ether.
0,6 kg. sodium.
12 kg commercial calcium chloride.

In addition, the cost of gas, electricity and water for distillation and vacuum drying.

After the above method of ether with cold extraction had been worked out and used in numerous series of investigations at the Carlsberg Laboratory we became acquainted with the carbonic tetrachloride method worked out by R. Seibriger (1913). Like ourselves Seibriger had seen, that petroleum ether owing to its heterogeneous composition was not a suitable means of extraction and sought to replace it with some homogeneous material; for this purpose he has chosen carbonic tetrachloride, which, as he states, has the advantage of not being inflammable. Seibriger crushes 40 gm. hops in a meat machine, uses the first 3 gm. to determine the amount of water, drying for 3 hours at 105°, mixes the rest well and extracts 10 gm. of the pulverised hops with 250 cc. carbonic tetrachloride. The mixture is shaken for 2 hours in an apparatus for this purpose, then the solution is filtered and titrated after Lintner's method.

The author remarks in his first publication (1913) that the values he obtains by using carbonic tetrachloride, are higher than

those obtained by trating the hops with petroleum ether on a water bath of 45—50° for 7 hours, and that these again are higher than the results obtained by shaking with cold petroleum ether. Nevertheless the results obtained by the different methods lie according to Seibriger within the permissible limits of error. The author thinks he has shown in this way, that the carbonic tetrachloride, which in his opinion extracts the resins more quickly and more uniformly than petroleum ether, can replace the latter.

It is a fact, that warm petroleum ether can dissolve the hard resin to a greater extent than cold petroleum ether. Since Seibriger's carbonic tetrachloride method gives still higher results, it seemed likely that this must be due to the carbonic tetrachloride also dissolving the hard resin — just as carbonic disulphide does. This is in fact the case, as we found on treating vaporised ether extracts of hops with respectively petroleum ether and carbonic tetrachloride. Whilst the former leaves a strongly yellow precipitate of hard resins, the carbonic tetrachloride dissolves everything with exception of a little colour substance (chlorophyll and the like.

In October 1913 Seibriger seems also to have recognised this, as he mentions in his paper »Ueber die Löslichkeit der Inhaltsstoffe des Hopfens«, that carbonic tetrachloride dissolves in addition to the α and β resins another substance which also reacts acid and is obviously a part of the γ resin which is insoluble in petroleum ether; as also that the β resin is changed on standing so that it becomes insoluble in petroleum ether, though still retaining its bitter taste and power to be dissolved by carbonic tetrachloride. Seibriger has thus in reality forsaken his original view, according to which weight was laid exclusively on the α and β resins; in the main, therefore, there is now agreement between the principles for the carbonic tetrachloride method and the method worked out by us. We have made comparisons between carbonic tetrachloride and ethyl ether as means of extraction and find, that the ether extracts considerably more quickly than carbonic tetrachloride. This will be seen from Table XVIII which shows two series of analyses of the same hops, the one treated by the carbonic tetrachloride method, the other by our method. Though the hops chosen contains only a small quan-

tity of resin, there is nevertheless a considerable difference in the results.

TABLE XVIII.

Carbonic tetrachloride method; 2 hours' extraction							
Sample	I	II	III	IV	V	VI	Average
% resin	5,4	5,2	5,4	5,6	5,4	5,6	5,4
Our method: 1 hour's extraction							
Sample	I	II	III	IV	V	VI	Average
% resin	6,7	6,8	6,7	6,6	6,7	6,7	6,7

This seems to show, that carbonic tetrachloride is not able to extract the resins completely under the conditions indicated in the method, as can also be seen on further extracting the hops by repeated shaking with carbonic tetrachloride. To decide the question further, that the difference in the results obtained by the two methods is only due to the fact, that the duration of the extraction proposed for the carbonic tetrachloride method is not sufficient, but not to the carbonic tetrachloride and ethyl ether dissolving different parts of the resins, we have treated the same quantity by weight, 0,75 gm., of the total resins with respectively 125 cc. carbonic tetrachloride and 125 cc. ether. As mentioned earlier the carbonic tetrachloride leaves behind a colouring matter; this was filtered off, dissolved in ether and showed neutral reaction. The resin solutions were then titrated and it proved, that both used 37,5 cc. 0,05 n. potassium hydroxyde solution for neutralisation. — We have also compared the two methods with regard to the hops tannic acid and Table XIX shows, that it is just as little permissible to extract moist hops with carbonic tatrachloride as with ethyl ether.

TABLE XIX.

(Brambling, 7 per cent water.)

Extracted with carbonic tetrachloride.				
	I	II	III	Aver.
Moist hops % resin (estimated from dry stuff). .	12,7	12,5	12,4	12,5
Dry hops % resin.	12,0	12,1	12,2	12,1

TABLE XIX (cont.).

Extracted with ethyl ether

	I	II	III	Aver.
Moist hops % resin (estimated from dry stuff).	14,3	14,3	14,4	14,3
Dry hops % resin	13,6	13,9	13,9	13,8

When we remember further, that carbonic tetrachloride is distinctly dearer than ethyl ether and not easier to recover, as also that in the carbonic tetrachloride method absolute alcohol must be added for the sake of the titration, there remains only this advantage of the method by contrast with ethyl ether, that carbonic tetrachloride is not inflammable. In cold extraction and using electrically heated apparatus in recovering the ether, this however is of very little importance.

We have also made a comparison with the ammonia method proposed by A. Nilson (1911). After Nilson the hops at the room temperature is repeatedly treated with ammonia of normality 1/20—1/10 or about 0,2 % NH_3. The total quantity of liquid filtered through the hops is made slightly acid with hydrochloric acid, by means of which the resins are precipitated in the form of a yellow powder, with which however some albumen is mixed. After the precipitate is formed, it is washed with water to remove the small quantity of acid remaining; then it is placed on a filter and treated with alcohol, which dissolves everything except the albumen present. The alcoholic solution is vaporised to dryness and the residue weighed.

With regard to the usefulness of the method it may be remarked, on the one hand, that the same quantity of resin cannot be dissolved with ammonia as with ether, and on the other, that ammonia does not exclusively dissolve the soft resins, since what is dissolved in ammonia cannot again be dissolved completely in petroleum ether. Add to this, that the rapid precipitation mentioned by Nilson did not occur in our experiments — the solution had rather an almost colloid nature — and that the subsequent filtration was extremely long and always gave a turbid filtrate, and it will be seen, that we cannot recommend the method.

We are of opinion, that this method, ether with cold ex-

traction, which we have used in a large number of investigations of hops .from the hops cultures of the Laboratory (up to the present over 5000 analyses), gives a convenient and trustworthy means of determining the resin contents of hops and thus in part its brewing value. We are convinced, that the use of this method will give direct, comparable results in analyses made by different analysts at different places, which cannot be said of the methods proposed earlier.

3. Résumé.

1. It proves that an error has been made hitherto in considering one of the resins of hops, the γ resin, as valueless; on the contrary, during the brewing process it both gives a taste to the wort and helps in the precipitation of the albumens.

2. It proves, that although the resins of hops which are all soluble in cold ethyl ether, are not all equally bitter and equally valuable — the relation between their bitterness can be expressed by the proportion $\alpha : \beta : \gamma = 10 : 7 : 4$ — yet the total quantity of resins extracted from the hops by means of cold ether and determined by titration is an approximately accurate expression of the bitterness value of hops.

3. It proves, that the analytical methods hitherto used, which are based on a separation of the so-called soft resins from the hard resin, rest upon an insecure basis and give misleading results.

4. A method is given (p. 132—133) for the extraction of the hops with cold ethyl ether and for the determination of the total quantity of the resins extracted by titration. By a practical method a titration factor has been found, which fully agrees with that calculated by Lintner and which makes it possible to express the bitterness value of the hops numerically.

5. A comparison has been made between the ethyl ether — cold extraction method worked out at the Carlsberg Laboratory and the carbonic tetrachloride method given by Seibriger in 1913. The results are discussed and it is shown, that the method of the Carlsberg Laboratory is to be preferred.

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JOHS. SCHMIDT:

INVESTIGATIONS ON HOPS

(*Humulus lupulus*, L.).

V. ON THE AROMA OF HOPS.

BY

JOHS. SCHMIDT.

I. Introductory Remarks.

It is a well known fact, that the lack of reliable methods for objective valuation of the quality of different hop samples is very keenly felt both by the practical brewer and by the scientific experimental investigator working with a view to improvement of hop-varieties or to the introduction of good varieties from other growing districts. The brewer, however, has still the traditional though frequently costly resource of taking the name and reputation of the place of origin as guaranteeing in some degree the quality of the hops, whereas in the case of scientific work, this is of course out of the question. In such work, it is of little use to know that hops, say from Saaz in Bohemia, have the reputation of being one of the best extant varieties — a reputation which must frequently be paid for at a high price — if it be true, as is generally asserted, that the Saaz hops, when transplanted and cultivated elsewhere, lose most of their prominent qualities. In a word, it is immediately obvious that all experimental work aiming at improvement of hop varieties can be but vague and uncertain as long as no objective method of valuation exists; to procure such a method is therefore one of the primary objects of any systematic endeavour to deal with the question.

It is thus hardly necessary to state that work of the Carlsberg Laboratory has, from the commencement of the hop investigations, been continually directed towards this end, more especially since the question is one of great practical importance.

It is a long and arduous task, however, and the solution of the problem demands the co-operation of many different branches of work, in addition to which, we have from the outset to reckon with the probability of opposition, if not from other quarters, at any rate from those economically interested in the maintenance of present conditions in the hop trade.

The qualities which a brewer takes into consideration when purchasing hops are numerous, and one buyer may lay most stress on one, another on another of these features. It will be undoubtedly agreed, however, that the lupulin-content and the aroma are points of primary importance in the brewer's estimate of quality, for which reason the work of the Laboratory has hitherto been especially directed towards these.

With regard to the first, the lupulin-content, a method for quantitative determination of this by means of extraction in cold ethyl-ether and subsequent titration of the solution with $^1/_{20}$ normal potassium hydroxide solution has been worked out at the Laboratory and described in a paper in the present series, by Ö. Winge and J. P. H. Jensen[1]). This method, by which the lupulin-content is obtained as a percentage of dry weight of the hops, has been largely employed in the Laboratory here for several years, both for qualitative valuation of the hops grown in our experimental garden and in analyses of trade samples made by the Laboratory in co-operation with various breweries. The method is easy and rapid, requiring but slight chemical training, and it is therefore to be hoped that it may also prove a serviceable aid to practical men.

This once given, the road towards improvement is now perfectly clear, inasmuch as regards an augmentation of the lupulin-content, and the work can be proceeded with in entire disregard of all the assertions in which the literature on hops is so prolific. It is otherwise, however, in the case of the second quality referred to: the aroma. There can be no question as to the fact that a certain high percentage of lupulin is desirable: the aroma, however is a quality, the valuation of which is to the fullest extent dependent upon a purely subjective estimate. Moreover, strange as it may seem, we cannot regard it as certain that the

[1]) Investigations on hops: (*Humulus lupulus L.*); IV. Ö. Winge and J. P. H. Jensen: A method for quantitative determination of resins in hops. [Comptes rendus des trav. du Labor. de Carlsberg, 11me vol. p. 116—147, 1914].

aroma present in a hop sample, when this is mixed with the wort for boiling, has any decisive influence on the flavour of the beer. I hope to return to this last point later on, and shall here consider the question of aroma in hops from a more botanical point of view.

Reference has already been made to the Saaz hop. This variety belongs, together with various others, to those which have for many years been held in high repute as possessing a fine aroma, and it cannot be denied that most samples of fresh, well handled hops from Saaz exhibit this quality in a high degree. I am unable to clearly define this aroma in words; should I attempt to do so, I could only call it »dry, without any rank or other sub-flavour« — this definition is, however, inadequate, and in our valuation lists, the word »Saaz-like« is frequently used to describe the aroma of one or another sample.

As already mentioned, it is generally believed in several hop-growing countries that a fine variety will, on being transplanted to other, and especially distant, localities, sooner or later lose most of its prominent qualities, the aroma in particular. This last is as a matter of fact, generally regarded as a very »volatile« (i. e. far from permanent) character, depending to a very high degree not merely upon climatic, but also upon purely local conditions (It will here suffice to mention the well-known fact that »Stadt« hops are frequently classed above »Bezirk« hops, this being actually the case, for instance, in Saaz).

A general assertion of this nature cannot, however, be immediately accepted, especially since the very first experiments in cultivation have shown, that in many cases a fine »variety« does not actually constitute a unit, either in botanical or in chemical respects. This is very clearly evidenced by our cultures, the leading principle of which is their being strictly kept as pure cultures. Even apart from this very essential point, however, the assertion is still too general, and the question must be formulated more precisely, for instance as follows: »To what extent and in what manner may a fine variety of hop, e. g. the Saaz, exhibit changes in its essential qualities when transplanted to other regions?« If we now write »Denmark« instead of »other regions« and add: »what alterations are apparent in these qualities when the fine variety in question is crossed with hardy Danish plants?« we have the very problem which I set myself

to study when commencing the hop investigations at the Carlsberg Laboratory in 1910

The various qualities investigated (lupulin-content, etc.) will be dealt with later on. For the present, I restrict myself to discussion of the aroma.

The hop is, as we know, a perennial plant, propagated not only by seeds but also to a great extent by vegetative process, through runners both below and above the ground. The lifetime of the individual plant is thus unlimited. In practical hop-growing, the vegetative method of propagation is exclusively or almost exclusively employed, the underground shoots (in Danish »Löberödder«, English »cuttings«, »cuts«, »sets«, German »Fechser«) being cut off during the resting period and planted, each then developing into a growth which is entirely identical with that from which the cutting in question was taken. Strictly speaking, this is not propagation at all, each plant thus obtained being only a portion of the original plant. And as a matter of fact, there may also exist young hop-gardens which are, so to speak pure cultures, i. e. consisting entirely of cuttings from one and the same plant selected on account of its good qualities. In older gardens this is rarely the case, as dropped seeds will, despite whatever care may be taken, occasion a growth of hybrid plants naturally exhibiting qualities other than those of the femals parent plant, and in this manner the purity of the stock will be impaired to a greater or lesser extent. In countries where the hops are generally allowed to pollinate and fructify (England. the United States, Belgium, Denmark) there will naturally be greater risk of adulteration than in those where seeding is as far as possible avoided (Germany. Austria); even here, however, it will scarcely be feasible in the long run to prevent such adulteration making its appearance among plants nominally propagated by the vegetative process alone. As a consequence of this, plants from a stock which may originally have been pure, and bearing a name derived from some quality peculiar to the »variety« from the locality of origin or from the name of the first grower, will gradually develop into a more or less evenly blended mixture of »clones«[1]) originating, as the

[1]) Dr. W. W. Stockberger, Department of Agriculture, Washington, has called my attention to the word »clone« (Gr. *κλών*: a twig or slip) introduced in 1903 by Webber, and defined by Geo. H. Shull (»Science«, 1912,

case may be, from a greater or lesser number of seedlings, i. e. individuals produced by fertilization.

Now if this be the case — and that it is so has been sufficiently proved by our cultivation experiments, *vide* p. 151 — then a natural consequence will be that a single »variety« of hop, in the ordinary acceptance of the term, may contain units differing widely both in botanical and chemical respects, and therefore also in quality. This again leads to the conclusion that in making exact comparative investigations with hops, whether from a botanical or from a chemical point of view, whether for practical or for scientific purposes, it is simply impossible to deal with mere »varieties« of hops as generally understood. All investigations of an exact nature in this field of work must be strictly based upon the resolution or breaking up of such »varieties« into their elements by means of pure cultures. In a word, it is necessary to work with clones and with these only, whether in comparing hops from different localities or from different years; any deviation from this fundamental principle may render investigations, otherwise exact enough, entirely valueless as evidence. A comparison of the lupulin-content in a series of different varieties from one year to another, such as one sees made from time to time (e. g. in »Allgemeine Brauer- und Hopfen-Zeitung«, No. 234, 1914) is thus at best of but slight interest, and may even produce results entirely misleading, when nothing is known as to the purity of the material. The application of carefully formulated chemical methods for the purpose of such investigations may best be compared with the pouring of a rare wine into dirty bottles.

From the foregoing it will be understood that the hop cultures of the Carlsberg Laboratory are, as mentioned on p. 151 maintained most strictly as pure cultures throughout. To do so demands a very considerable amount of work, necessitating as it does the removal of all shoots both above and below ground, in order to prevent »infection« of one clone by another, while

p. 183) as follows: »Clone, a group of individuals traceable through asexual reproductions (including parthenogenesis when unaccompanied by genotypic segregation) to a single ancestral zygote, or else perpetually asexual«. I would suggest that the word be adopted into the terminology relating to hops, where such a term is actually needed. A hop-clone would thus be all those plants derived from the same seedling by vegetative propagation, a clone-plant being any single plant belonging to the clone.

for the same reason, all seedlings growing up between the parent plants must be sought out and eliminated.

The experiments hereinafter described were commenced in 1911, and are still in progress. In the season of 1911, our experimental garden already contained a selection of the most important continental and English varieties of hops, obtained through the courtesy of experimental stations and private persons in the respective localities; this material has since been largely increased.

Each individual plant with its clone-plants has its own catalogue number, and is entered by this in the journals kept for each year, in which are noted all such data as have been obtained by observation of the separate plants and their separately gathered crop.

II. The Experiments made.

The question before us, as regards aroma, was as follows:

1) To what extent may the aroma in clones of fine foreign varieties of hops be changed on cultivation in Denmark?

2) To what extent is the aroma transferred to seedlings raised by crossing female plants of fine foreign varieties with wild-grown Danish hops?

My idea was to employ Saaz hops for the purposes of the experiment, as it would be interesting to try that fine variety in this country, and as there exists, so far as I am aware, no Danish hop with an aroma resembling that of the typical Saaz. The variety in question is also well suited to the purpose, inasmuch as it thrives excellently here, and is early-flowering (during the years we have had it in our garden is has been ready for picking by the first half of September).

The result of my observations of Saaz plants cultivated during the years 1911, 1912, 1913, 1914 in our experimental garden was, that the plants in question had, according to my personal judgement — based on comparison with samples of Saaz hops grown in the Saaz district — preserved their characteristic aroma throughout these years, the appearance of the hops, etc. being also the same. We have, moreover, found perfectly typical Saaz aroma in plants received from Lorraine and stated to have been originally introduced there from Saaz in Bohemia. With regard to the plants derived from Saaz hops

crossed with Danish male plants, it is, in my opinion, possible in several cases to assert with perfect certainty that the Saaz aroma is present; in most cases, however, where a difference in degree — »a diluted« Saaz aroma, so to speak — is perhaps noticeable, I dare say nothing definite. After all, the difference between Saaz aroma and that of many other plants, Danish included, is not sufficiently great. We have here to face the regrettable fact that no exact method exists by which to solve the problem, and in order to arrive at a reliable result, I have been compelled to try another way.

Among the plants procured, as already mentioned, in 1911 from the principal hop-growing districts of Europe, were also included some few specimens of American hops, sent from that country by Hr. N. Hjelte Claussen, then resident in Chicago, now in Copenhagen. The hops obtained from these plants had an aroma so entirely different from that of any variety of hop then known to me, that I considered them, for that reason, far better suited to the purpose of the investigations which I had in view than any available European variety. The aroma was so peculiar, and so unlike what we in Europe are accustomed to meet with in hops, that to the uninitiated it would at first seem hardly possible that using such hops a fine beer could be produced, which as a matter of fact is the case.

The aroma in question is, I should say, rather much like that of turpentine, with a touch of something faintly resembling the aromatic scent associated with *Ruta graveolens*, a plant from the south of Europe belonging to the family *Rutaceæ*.

I have subsequently, however, on going through a number of samples grown in America, learned that many of the American varieties have a similar aroma, very different from that of European hops. I use the term »American« aroma here in contrast to »European« aroma without in any way wishing to suggest that the former should be of poorer quality than the latter.

The plants in question were sent to me under two different names, viz: »Oregon Cluster«, said to come from California, and »New York Spaulding English Cluster«, stated as having been grown in Waterville, New York State. Both plants produced hops having the distinctive »American« aroma, this being, however, most prominent in the »Oregon Cluster«. The hops themselves were, moreover, different in appearance from any European hops I

know, especially by their pale green colour and by the fact that the stipules were distinctly mucronate.

The first question was concerned with the constancy of the aroma in the plants imported from America in 1911, Nos. 60 (Oregon Cluster) and 99 (New York Spaulding English Cluster). This question can at once and without restriction be answered in the affirmative; the clones of both plants have, during each of the years 1911—1914 inclusive, produced hops with distinctly »American« aroma, this being, moreover, so conspicuous that it was possible to immediately recognise a single hop among hundreds of those of the European sorts, whether Danish, English, German etc. There is no indication of any diminution in the strength of the »American« aroma during the four years' stay in Denmark: the cultivation will, however, be continued, so that observations as to this may be made throughout a further series of years.

It may be added that in November 1914, we compared the different lots of hops obtained here from the Oregon Cluster No. 60 in the years 1912, 1913 and 1914 respectively, and found that while the sample from 1914 still possessed in a very high degree the characteristic »American« aroma, this had, in the 1913 sample almost, and in that from 1912 entirely disappeared.

We have now to consider the question as to transmission of the aroma to the seedlings. Both No. 60 (Oregon Cluster) and No. 99 (N. Y. Spaulding English Cluster) were crossed with a male plant, a pure culture of which is to be found in our experimental garden. It is originally a wild plant, which I collected in the spring of 1911 at Færgelunden near Jægerspris, in the north of Sealand. No. 60 was pollinated in 1912, and the resultant seeds sown in the spring of 1913. Of the seedlings thus obtained, several flowered the same year, others not until the following (1914). Thus the crop from the first named seedlings could be judged both in 1913 and 1914 (cf. Table I), the last not until 1914 (cf. Table II) No. 99 was pollinated in 1913. A number of the seedlings flowered in 1914, so that their crop could be judged the same year (cf. Table III).

The examination of the crop from the different seedlings was carried out, both in 1913 and 1914, by the writer and the Laboratory Assistant, mag. sc. Ö. Winge, the estimates being made by each independently of the other. The results will be

TABLE I A.

Transmission of »American« aroma to seedlings raised by crossing ♀ No. 60 (Oregon Cluster) and ♂ No. 4 (Denmark). Valuation of same seedlings in 1913 and 1914.

No. of seedling	1913				1914			
	Development of hops (1—3)	»American flavour« (Strength 1—3) Estimat. by Schmidt	»American flavour« (Strength 1—3) Estimat. by Winge	Remarks	Development of hops (1—3)	»American flavour« (Strength 1—3) Estimat. by Schmidt	»American flavour« (Strength 1—3) Estimat. by Winge	Remarks
1190	2 1/4	3	3		undeveloped	—	—	indeterminable
1191	1 3/4	2	2		1	2	2	
1192	1	none	1		1	none	none	
1193	2 1/4	3	3		2 1/4	3	3	
1194	2 1/4	2	2		2 3/4	2	1 1/2	
1195	1 1/2	none	none		2 1/2	1	1	
1196	1 3/4	none	none		2 1/4	1/2	1	
1197	2 1/4	2	2		3	3	2 1/2	
1198	2	3	3		—	—	—	
1199	2 1/2	3	3		3	3	3	
1200	2	1	none		2 3/4	1/2	1 1/2	
1201	1 1/2	none	none		2 3/4	none	none	
1202	1 1/2	2	3		2 1/4	3	3	
1203	1 3/4	2	2		1 1/2	1	2	
1204	1 1/2	1	none		2	1	2	
1205	2	none	none		3	none	none	
1206	2	3	2		—	—	—	
1207	1 3/4	2	2		1	1	1	
1208	2 1/2	1	1		2 1/2	1	1	
1209	1	none	none		1 1/2	1/2	none	
1210	2	2	2		2	3	3	
1211	2 1/2	none	none	Specially rich in lupulin	1 3/4	none	none	Specially rich in lupulin
1212	1 3/4	1	1		2 1/2	1	1	

TABLE I B.

Same experiment as in Table I A: Summary.

Estimated by	1913				1914			
	Schmidt		Winge		Schmidt		Winge	
No. of seedlings	23	»	23	»	20	»	20	»
Strong »American flavour«	5	22 %	5	22 %	5	25 %	4	20 %
Less strong » »	11	48 %	10	43 %	11	55 %	11	55 %
No »	7	30 %	8	35 %	4	20 %	5	25 %

TABLE II A.

Transmission of »American« appearance and aroma to seedlings raised by crossing ♀ Nr. 60 (Oregon Cluster) and ♂ Nr. 4 (Denmark).
Estimated in 1914.

No. of seedling	Development of hops (1—3)	»American« appearance?		»American« flavour?		Remarks
		Estimat. by Schmidt	Estimat. by Winge	Estimat. by Schmidt	Estimat. by Winge	
1193	2 1/4	?	?	Yes	Yes	
1194	2 1/4	?	Yes	Yes	Yes	
1195	2 1/4	Yes	Yes	?	?	
1196	1 3/4	—	—	?	?	
1197	2 3/4	Yes	Yes	Yes	Yes	
1199	3	Yes	Yes	Yes	Yes	
1200	2 1/4	Yes	Yes	?	?	
1201	2 3/4	Yes	Yes	No	?	
1202	2 1/4	—	—	Yes	Yes	
1203	1 1/2	Yes	Yes	Yes	Yes	
1204	2	?	?	?	Yes	
1205	3	No	No	No	No	
1207	1 3/4	—	—	Yes	Yes	
1208	1 3/4	Yes	Yes	Yes	?	
1209	1 1/2	Yes	Yes	Yes	Yes	
1210	2	—	?	Yes	Yes	
1211	1 3/4	—	—	No	No	
1212	2 1/2	No	No	Yes	Yes	
D 1	3	Yes	Yes	Yes	Yes	
D 2	3	Yes	Yes	Yes	Yes	
D 3	2 3/4	Yes	?	Yes	Yes	
D 4	2 1/2	Yes	Yes	Yes	Yes	
D 5	2 1/2	Yes	Yes	Yes	Yes	
D 6	2 3/4	No	?	Yes	Yes	Only noticeable in some few of the hops
D 7	2	?	?	Yes	Yes	
D 8	2 1/4	Yes	Yes	No	No	
D 9	2 1/4	Yes	Yes	Yes	Yes	
D 10	2 1/2	?	?	No	?	
D 11	2 3/4	?	?	Yes	Yes	
D 12	2 1/4	Yes	Yes	Yes	No	
D 13	2	?	?	No	No	
D 14	1 3/4	?	?	Yes	Yes	
D 15	2 1/4	Yes	Yes	?	No	
D 16	2 1/4	Yes	Yes	Yes	Yes	
D 17	2 1/2	Yes	Yes	?	No	
D 19	2 1/2	No	No	No	No	
D 20	2	Yes	Yes	?	Yes	
D 21	2 1/2	?	?	Yes	Yes	

— indicates that the sample in question was not found suitable for consideration (owing to insufficient development or fungus attack)

Table II A (Continuation).

No. of seedling	Development of hops (1—3)	»American« appearance?		»American« flavour?		Remarks
		Estimat. by Schmidt	Estimat. by Winge	Estimat. by Schmidt	Estimat. by Winge	
D 22	1 3/4	?	?	No	No	
D 23	1 1/2	—	—	Yes	Yes	
D 24	2 1/2	Yes	Yes	No	No	
D 25	2	?	Yes	No	?	
D 43	2	?	?	Yes	Yes	
D 44	2	?	?	Yes	Yes	
D 45	1 3/4	?	?	Yes	Yes	

TABLE II B.

Same experiment as in Table II A: Summary.

Estimated by	Appearance of hops				Flavour			
	Schmidt		Winge		Schmidt		Winge	
No. of seedlings	38	»	39	»	45	»	45	»
Distinctly »American«.	21	55 %	22	56 %	28	62 %	28	62 %
Doubtful.....	13	34 %	14	36 %	7	16 %	7	16 %
Not »American«	4	11 %	3	8 %	10	22 %	10	22 %

found in Tables I—III. The following points should here be noted: Only samples in which the lupulin was ripe were judged. The development (i. e. size) of the hops, is stated, on a purely individual estimate, as from 1—3, the latter figure indicating fully, the former poorly developed hops. Tables II and III are merely concerned with the question as to whether the different samples exhibited »American« aroma and appearance; in Table I however, an attempt has further been made to classify the strength of the »American« aroma on a scale of 1—3, where 3 indicates very strong, and 1 only slightly noticeable »American« aroma. Section B. gives a summary of the results in each table, with percentages.

On the whole, the results of the valuations made by the two different investigators agree very well, varying mostly in cases where it is a question of determining whether the »American« aroma is very faintly noticeable in a sample or not present at

TABLE III A.

Transmission of »American« appearance and aroma to seedlings raised by crossing ♀ No. 99 (New York Spaulding English Cluster and ♂ No. 4 (Denmark). Estimated in 1914.

No. of seedling	Development of hops (1—3)	»American« appearance?		»American« flavour?		Remarks
		Estimat. by Schmidt	Estimat. by Winge	Estimat. by Schmidt	Estimat. by Winge	
D 26	2¼	Yes	Yes	No	No	— indicates that the sample in question was not found suitable for consideration (owing to insufficient development or fungus attack)
D 27	2½	No	?	?	Yes	
D 28	3	Yes	?	Yes	Yes	
D 29	2¾	?	?	No	No	
D 30	3	No	No	Yes	Yes	
D 32	1¾	—	—	Yes	Yes	
D 33	1¾	Yes	Yes	No	No	
D 34	2	?	?	No	No	
D 36	2¾	Yes	Yes	Yes	Yes	
D 38	2¼	?	?	Yes	Yes	
D 39	2¾	Yes	Yes	Yes	Yes	
D 40	2	No	No	No	No	
D 41	2½	?	?	—	No	
D 42	1½	Yes	—	Yes	—	

TABLE III B.

Same experiment as in Table III A: Summary.

Estimated by	Appearance of hops				Flavour			
	Schmidt		Winge		Schmidt		Winge	
No. of seedlings	13	»	»	»	13	»	13	»
Distinctly »American«.	6	47 %	4	33 %	7	54 %	7	54 %
Doubtful	4	30 %	6	50 %	1	7 %	0	0 %
Not »American«	3	23 %	2	17 %	5	39 %	6	46 %

all. Had the question been formulated in a slightly different manner, say as follows: »Is strong »American« aroma present?« there would probably have been no difference of opinion at all.

As regards No. 60, Oregon Cluster, we see from Table I, that »American« aroma was found in about ¾ of the offspring, and lacking in the remaining fourth; this applies to the crop from these plants both in 1913 and 1914. In Table II, which embraces a larger number of specimens, similarly offspring of No. 60, distinction is made between 1) distinct »American«

aroma, 2) no »American« aroma, and 3) doubtful if any »American« aroma present. If we now presume, as is probably the case, that the category »doubtful« includes specimens which by right belong to (1) as well as to (3), then we arrive at the same result as in Table I, viz: that the »American« aroma is transmitted to abt. 3/4 of the offspring. The offspring of No. 99, however (New York Spaulding English Cluster) show somewhat lower figures, only a little over half the plants having »American« aroma. The reason of this difference I am unable to explain: it may possibly to some extent be due to the small number of plants examined (only 13 in all), possibly also to the fact that the »American« aroma was hardly so markedly evident in the parent plant No. 99 as in No. 60.

A point of considerable interest is the appearance of the hops in the different offspring plants (cf. Tables II and III). These plants, derived from the same male and female parents, differ one from another to such a degree that some of them would be declared by any hop merchant to belong to entirely different varieties — which would, moreover, be perfectly correct. As will be seen from the tables, we have in most cases classified the appearance of the hops as »American« (cf. p. 156) in others there is no trace of this, while some few again present an appearance entirely different from that of any hops known to us. From Table II A, it will be seen (cf. plants Nos. 1199, 1205, 1212 and D. 8, etc.) that typical »American« appearance may be associated with the presence or with the absence of »American« aroma, whereas on the other hand, hops with nothing at all »American« in their appearance, may possess the »American« aroma in a very high degree, or lack it entirely.

It is thus evident from the foregoing, that the »American« aroma is a quality which may be transmitted to the offspring plants in the same way as other more »tangible« characters, and probably follows here the same laws and arithmetical conditions as apply to these.

As to how far the »American« aroma and appearance may be transmitted from the male parent to the offspring, I am unable to determine at the moment. We have, however, with this point in view, procured seeds in 1914 by crossing American male plants with European females, so that I hope in 1915 to

be able to make this matter clear, the question being one of considerable interest. What we have to consider is the possibility of transmission of qualities to female offspring from the male parent plant, which in itself is of course phænotypically lacking in such qualities.

In all the experiments here referred to I have been assisted by Hr. mag. sc. Ö. Winge, to whom I take this opportunity of expressing my best thanks.

III. Summary.

1) In all districts where the male hops have not been entirely eradicated — and has this anywhere been accomplished? — large stocks of hops will, in the long run, inevitably become adulterated. Many, and possibly all, of the older »varieties« or »sorts«, in the ordinary acceptance of these terms, will therefore consist of several different clones (*vide* note p. 152).

2) In exact investigations as to hops, whether of botanical or chemical, practical or scientific nature, the work should be based upon clones, as otherwise the results may be liable to justifiable criticism.

3) Among the hop plants cultivated in the experimental garden of the Carlsberg Laboratory, there were in 1911, two American plants (labelled »Oregon Cluster« and »New York Spaulding English Cluster«) the hops of which exhibited a very peculiar turpentine-like aroma, so widely different from that of all European varieties, that a single hop could at once be recognised by the smell among hundreds of others. Cultivation experiments made during the years 1911—1914 showed that this aroma remained apparently constant in our climate in these two plants and their clones.

Crossing experiments made in 1912—1914 with the two American plants and Danish males showed that the turpentine-like aroma was transmitted to between $^1/_2$ and $^3/_4$ of the offspring plants, without regard to whether the hops themselves retained the appearance peculiar to those of the mother plant.

4) From the experiments already mentioned, as well as from several others made with European varieties (e. g. those from Saaz in Bohemia) it would seem that the aroma of hops is not to be regarded as so »volatile« a character, or so entirely due to purely local conditions, as has generally been believed.

Postscriptum.

Since writing the above, I have received a most interesting and very important paper by E. S. Salmon, dealing in part with exactly the same question as the present work (E. S. Salmon: Notes on Hops, 1912—1914, University of London, County Councils of Kent and Surrey, South-Eastern Agricultural College, Wye). The work in question is a report of the crossing experiments made by the writer in the experimental hop-garden at the Agricultural College at Wye, in Kent (England). E. S. Salmon has (l. c. p. 23—24 and 29), investigated 8 plants obtained through pollination of a female Oregon Cluster by English male plants, and found, that: »Some of the seedlings (4) have inherited this (i. e. the Oregon) flavour, either apparently unaltered (2) or more or less modified (2): other seedlings (4) however, produce hops which show no trace whatever of the Oregon parentage as regards their aroma«.

From this it will be seen that there is the most excellent agreement between Mr. Salmon's results and ours (cf. the fact that we found the peculiar aroma to be transmitted to between $^1/_2$ and $^3/_4$ of the offspring, with Mr. Salmon's experiments, where half the offspring retained the aroma in question). I may add that as far as the lupulin-content is concerned, we have, like Mr. Salmon, found very high percentages in the offspring of the Oregon Cluster No 60. Altogether, these two series of investigations, carried out independently in England and Denmark respectively, exhibit remarkable uniformity of results, and the discoveries thus made appear to promise well for the systematic improvement of hops. Mr. Salmon's report, moreover, shows that good results have already been obtained in this respect.

Printed January 30, 1915.

JOHS. SCHMIDT:

INVESTIGATIONS ON HOPS

(*Humulus lupulus*, L.).

VI. ON THE AMOUNT OF LUPULIN IN PLANTS RAISED BY CROSSING.

BY

JOHS. SCHMIDT.

1. Introduction.

SINCE 1911, a selection of the most important sorts of foreign hops have been cultivated in the experimental garden at Carlsberg Laboratory. The object of this cultivation was twofold, viz: 1) to test such older sorts in the Danish climate (»Investigations as to the constancy of valuable qualities in older clones«)[1]), and 2) to cross them with Danish and other male plants, endeavouring thus to produce new good sorts (»Formation of new clones with combinations of valuable qualities«).

The qualities which have hitherto been taken into consideration are as follows:

1) Time of flowering (»earliness«).
2) Productivity (No. and weight of hops per bine).
3) Lupulin content (stated as a percentage of the dry weight of the hops).
4) Aroma.

The qualities mentioned under Heads 1) and 2) are directly measurable by simple counting and weighing; the lupulin content, however, is determined according to the method described by Ö. Winge and J. P. H. Jensen[2]) by extraction of the dried

[1]) With reference to the term »clone«, *vide* »Investigations on hops (*Humulus lupulus*, L.)«. — V. On the aroma of hops, by Johs. Schmidt. (Comptes rendus du Laboratoire de Carlsberg, Vol. 11, p. 149—163, Copenhagen 1915).

[2]) »Investigations on Hops (*Humulus lupulus*, L)«. — IV. A method for quantitative determination of resins in hops, by Ö. Winge and J. P. H. Jensen. (Ibid, Vol. 11, p. 116—147, Copenhagen 1914).

hops with cold ethyl-ether, and titration of the solution with 0,05 n. potassium hydroxide solution, the result being stated as a percentage of the dry weight of the hops.

Finally, as regards the aroma, there exists no practicable method for objective determination in small samples, and we are here forced to content ourselves with a mere subjective estimate. In this connection I may refer to a report recently published regarding the peculiar turpentine-like aroma in certain American hop plants, and the constancy of same in clones, and in plants raised by crossing[1]).

It need hardly be said that investigations of such a character as those here planned demand a considerable length of time, and as a matter of fact, none of our experiments, either under Head 1) or Head 2) have as yet been brought to a conclusion. The former will not be dealt with here at all; I wish, however, even at this early date, to make some preliminary statements regarding such results as have hitherto been obtained from our first crossing experiment in 1911.

My reason for so doing is in the first place the fact that the experiment is already partially concluded, as we have unfortunately been obliged, owing to want of ground space, to root up the great majority of the seedlings after a first inspection. And in the second place, even this single inspection of the very considerable number of seedlings here concerned already appears to furnish in several respects a useful idea as to what may generally be expected from such crossing experiments with hops.

In any hop garden where male plants are present, spontaneous crossing will take place, giving rise to clones having other qualities than those of the mother plants. There is in my opinion little doubt that most new sorts or varieties are and have been produced by crossing[2]). Up to the present, comparatively little has been accomplished in the way of methodical crossing of hops, all the important varieties at present cultivated having

[1]) »Investigations on Hops (*Humulus lupulus*, L.). — V. On the aroma of hops, by Johs. Schmidt. (Ibid. Vol. 11, p. 149—163, Copenhagen 1915).

[2]) The various statements published as to the appearance of new sorts by vegetative means (»spontaneous variation« of an existing clone) appear somewhat improbable. In any case, if such statements are to be accepted, they must be based upon no slight degree of competence in the observer, as well as upon such pure culture as is hardly to be found in any hop garden with purely commercial ends in view (cf. Johs. Schmidt, l. c. 1915, p. 152).

apparently originated from casual finds of seedlings produced by accidental crossing. This does not, however, in any way imply that there should be no prospect of obtaining considerable results by systematic crossing of hops: on the contrary, it would seem that there are excellent chances of success in such work. In contrast to most of our cultivated plants, the hop is perennial, and is propagated vegetatively, which fact in itself increases to a very high degree the possibility of »fixing« and retaining the most valuable qualities produced by crossing.

We learn from works on the subject[1]) that crossing experiments with hops have, at least occasionally, been carried out in most of the hop-growing countries since the conclusion of the last century, and good results have probably been obtained in several cases, albeit perhaps for the most part of purely local character. There seems to have been more done in England than in the two other great European hop-growing countries (Germany and Austria), which fact has perhaps some connection with the view generally accepted in the latter countries that male plants should be exterminated as far as possible, as the presence of seeds in hops is detrimental to the quality of the beer. (In England, on the other hand, male plants are especially cultivated in the hop gardens, the presence of seeds being here usually regarded as necessary of the proper development of the hops).

Among the crossing experiments which have been made on the Continent may be mentioned, as particularly worthy of note, the work commenced in 1898 by Th. Remy[2]), of Berlin, which appears to have been arranged in so excellent and thoroughly scientific a manner, that the premature discontinuation of the experiment owing to the removal of the writer in question from Berlin before final results had been obtained is very much to be regretted. Remy classed his seedlings both in regard to content of lupulin and of tannins. Another experiment which should be mentioned is that made by C. Fruwirth[3]) in 1900, from which it appears that the average yield of the seedlings

[1]) A survey of this work will be found in C. Fruwirth: Die Züchtung der landwirtschaftlichen Kulturpflanzen, Bd. III, pp. 76—79, Berlin 1910 (Parey).

[2]) Th. Remy: Die Züchtung im Dienste des Hopfenbaues (Wochenschrift für Brauerei No. 36, p. 402 etc. Berlin 1903).

[3]) l. c. p. 79.

was greater than that of vegetatively produced plants of the same variety; viz: early Steiermark.

More important, it would seem, are the crossing experiments which have been made in England. Especially worthy of note is the work which has been carried out in this respect since 1906 by E. S. Salmon, of the Agricultural College at Wye, in the county of Kent. In order to give some idea as to the practical nature of the methods here employed, I take the liberty of quoting the following passages from the introduction to Mr. E. S. Salmon's last report[1]):

»During the season of 1913 visits were paid to the College hop-nursery by over twenty hop-growers, who selected for trial in their hop-gardens several of the most promising-looking Seedlings. In all, 2,830 »cuts« from twenty-three different varieties were distributed to twenty-nine hop-growers farming at Brenchley, Harbledown, Selling, Paddock Wood, Dover, Woodnesborough, Hawkhurst, Leigh, Canterbury, Chilham, Goudhurst, Cranbrook, Barming, Yalding, Marden, Horton Kirby, Wateringbury, in Kent; Bodiam, Midhurst, Battle, Ticehurst, Uckfield, in Sussex.«

»In addition to the above, 202 »rooted sets« of ten varieties have been planted in the Experimental Hop-garden at the College Research Station at East Malling.«

»The college hop-nursery is open to inspection at any time to hop-growers in Kent, Surrey or Sussex.«

»As in previous seasons, samples of hops were collected from the most promising seedling and other varieties and dried and pressed into box samples for report as to flavour, etc., by experts in the Borough.« etc etc.

In addition, chemical analyses have been made as to the soft resins content of several seedlings, their degree of immunity against parasites has been tested, etc.

Finally it may be mentioned, that no methodical crossing experiments have hitherto been made with hops in Denmark, although, as will subsequently be seen, the Danish hops appear to stand more in need of improvement than those of any other country. The reason of this is doubtless to be sought in the lack of interest generally evinced in hop growing, and the

[1]) E. S. Salmon: Notes on Hops, 1913—14, p. 17 (Journal of S. E. Agricultural College, 1914).

poor state to which the industry in this country has declined of late years.

We may now proceed to consider the crossing experiments carried out in 1911.

2. The experiments.

The crossings were made in the experimental garden of the Carlsberg Laboratory in July and August 1911. The mother plants consisted of a large number of fine varieties from various important hop districts in Europe. Having then no cultures of male plants in the experimental garden, pollen was collected from wild plants in a wood in the north of Sealand (Færgelunden at Jægerspris). The pollen was taken as far as possible only from a single plant (»♂ No. 3«) the bines of which covered the surface of a bush: we have, however, no sort of guarantee that these all in reality belonged to one and the same clone[1]). It is thus not absolutely certain that all the seedlings obtained by crossing with »♂ No. 3« were derived from the same father. As regards the flowering time of »♂ No. 3« it may be mentioned that this was at its height (full bloom) in mid-August, and the plant must thus probably be classed as intermediate. In addition to ♂ Nr. 3, an earlier male (♂ No. 1) was also employed during the experiments in 1911, this plant having been found growing up a fence near the Laboratory. It consisted, however, of but a single bine, and furnished only pollen for one experiment. As to its flowering time, the records state that by the 31st July 1911 it had already passed the middle stage of full bloom.

The pollination was carried out in the following manner: — The female inflorescences were, while still in bud, enclosed in paper bags, which were fastened round flowering branches. The flowers then opened while still thus enclosed, by which means promiscuous pollination was avoided. The desired pollination was subsequently effected by removing the bags for a moment, and shaking pollen over the styles with a light brush, which done, the bags were replaced. Not until the young inflorescences had commenced to increase conspicuously in size, and the styles had fallen off, were the bags finally removed, a piece of bast being tied in a knot about the stalk to mark their place in the shoots.

[1]) The plant marked »♂ No. 4« was isolated from »♂ No. 3«.

The pollinated hops were separately picked, and as a rule not until a week after the others. For this reason the seeds collected showed a high percentage of germination. As a result, however, innumerable seedlings made their appearance next year throughout the whole of the nursery grounds, necessitating a great deal of weeding, and incidentally proving how difficult it is to keep a hop garden perfectly »clean«.

The seeds were sown in the spring of 1912, partly under glass frames, and partly in the open air The seedlings were transplanted in early summer, some in a glass-house, and others in the open air. A large number both of indoor and outdoor plants flowered the same year, so that we were able to get rid of a lot of male plants at once. The seedlings were finally, in the autumn of 1912, assigned to their definite places in a hop garden laid out close to the Laboratory according to the system generally adopted in Kent, save that the plants were set somewhat closer than usual, and trained vertically up from the breast-wire to the top-wire. Each plant had, as far as possible, two bines.

The investigations here mentioned refer to the season 1913.

After the picking in that year, analyses were made as to the lupulin content of all samples (which had been pollinated by the presence of male plants in the garden). A lupulin content of 14 % was taken as the limit for selection, only those over this figure being preserved for further investigation. The number of those which passed this preliminary test was 51, or about 7 % of the total number. The remaining female plants, 693 in all, were, with the male plants, cleared away in the autumn of 1913, owing to want of space.

3. The results of the crossing experiments.

Table I gives a graphical survey of the results of all lupulin analyses for two lots of seedlings raised by crossing two German female plants, viz: »Spalter mittel No. 16« and »Bayrisch spät No. 1« with »♂ No. 3«. The first lot consisted of 87, the second of 76 sister plants.

It will be seen that the lupulin percentages found vary greatly within each of the two groups. In the former, for instance, we find that the percentage ranges from about 8 to about 20, and in the latter from about 7 to about 17. Only few individuals, however, exhibit these extreme values, by far the greater part

TABLE I.

Crossing experiments 1911. — Lupulin content in offspring, in 1913, graphical view.

Lupulin percentage	No. of daughter plants
	»Spalter mittel No. 16« × »♂ No. 3«
20	o
19	
18	o o
17	o o o
16	o
15	o
14	o o o o o o o o o o
13	o o o o o o o o o o o o o o o o o o
12	o o o o o o o o o o o o o o o o
11	o o o o o o o o o o o o o o o o o
10	o o o o o o o o o o o o o o
9	o o o o o o o o
8	o.
	»Bayrisch spät No. 1« × »♂ No. 3«
17	o
16	o o
15	o o o o o o
14	o o o o o o o o o o o o o o o o o o
13	o o o o o o o o o o
12	o o o o o o o o o o o
11	o o o o o o o o o o o o
10	o o o o o o o o
9	o o o o o o o
8	o
7	o

in both cases occupying intermediate positions, the variates grouping themselves most closely about 12 % or so. In the first case, the average lupulin percentage of all specimens amounts to about 11.9, and in the second, about 12.3.

The same grouping as found in the examples above quoted is further encountered, as regards the lupulin content, in all the other crossing experiments; i. e. the majority of specimens exhibiting percentages approaching the average figures for all, the number of individuals decreasing as we near the extreme

values for maximum and minimum (the extreme plus and minus variates). We thus find that the lupulin content in a group of sister plants exhibits, as might be expected, a distribution corresponding to that found for all other mensurable qualitites, whether of morphological or physiological nature.

Table II shows the lupulin contents for all the seedlings. It should be noted that the mother plants in question were isolated from the consignments of cuttings forwarded to the Laboratory at different times, under the names of the respective varieties, from various hop districts, and subsequently propagated as pure cultures. The table does not include all the separate results of analyses — in all 744 — but only the average, maximum and minimum values for the different sister-groups.

Table III shows the results so arranged as to include the lupulin percentages of the mother plants, with a group arrangement according to the origin of the latter, distinction being made between three such groups: 1) German and Austrian plants, 2) English, and 3) Danish plants. The lupulin content of the seedlings refers only to the season 1913, the figures given for the mother plants however, are averaged from the percentages found in 1912, 1913 and 1914[1]).

On comparing the figures for offspring and mother plant, as shown in the table, it is at once evident that the average lupulin content in the former is far inferior to that of the latter. Save for some few exceptions (Danish plants) which will be subsequently referred to, this feature is apparent throughout, and to such a remarkable degree — the difference amounting to several per cent, — as to leave no doubt regarding the actual existence of the peculiarity in question. Throughout all our crossing experiments, the average lupulin content of the offspring shows a decrease. What this means may be illustrated by the following hypothetical case. Given a hop garden, containing say 31 specimens of the clone mentioned in Table II as »Brambling No. 40«, and another garden with the 31 seedlings obtained

[1]) * affixed to the figures for lupulin percentage of mother plants denotes that the plant in question was not grown in the open air, but in a glass-house (cold house) in the experimental garden of the Laboratory. Comparative investigations have, however, shown that the percentage of lupulin is not influenced by this, save in the case of certain plants, for the most part American, which do not ripen at all in our climate.

TABLE II.

Crossing experiments 1911. — Lupulin content 1913 in offspring.

Parents			Offspring			
♀		♂	No. of plants	Lupulin percentage		
				Average	Maximum	Minimum
	No.	No.				
Spalter mittel	136	3	29	10.1	13.5	6.5
»	16	3	87	11.9	19.7	7.7
»	32	3	25	10.6	16.0	5.7
»	32	1	14	11.2	14.9	8.6
Bayrisch spät	9	3	13	9.6	12.5	6.6
»	1	3	76	12.3	17.2	7.4
Braunschweiger . . .	18	?	23	10.2	17.3	5.5
Saaz	130	3	31	9.3	11.9	5.7
»	2	3	12	10.0	13.3	6.9
»	132	3	42	9.1	14.8	5.7
»	73	3	20	9.8	15.0	6.7
» roter	75	3	33	11.4	17.1	8.4
Golding	44	?	18	10.0	13.1	5.7
»	93	?	34	10.1	15.3	5.5
»	187	3	30	10.2	13.6	8.1
»	36	3	33	11.7	15.5	8.5
»	36	4	17	11.2	14.5	9.0
Brambling	64	?	23	9.4	11.7	6.3
»	40	4	31	9.6	13.4	6.1
»	40	3	19	8.9	12.6	5.9
Fuggles	175	?	30	11.0	15.9	8.0
»	38	4	6	11.8	13.6	10.1
Early Hobbs	61	3	21	10.1	14.6	6.6
Early Bird	167	3	4	7.4	8.3	6.0
Asperup	207	?	11	7.8	10.0	5.2
»	210	3	1	7.3	7.3	7.3
Graa	41	4	35	8.8	13.3	4.9
Grøn	199	3	26	8.4	15.3	3.8

TABLE III.

Crossing experiments 1911. — Lupulin content of all mother plants and offspring.

	Parents			Offspring		
	♂	♀	Lupulin percentage Average of 1912-1913-1914	Lupulin percentage Average 1913	Lupulin percentage Maximum 1913	No. of plants
	No.	No.				
1. German and Austrian plants.	3	Spalter mittel . . . 136	11.2	10.1	13.5	29
	3	» . . . 16	13.2	11.9	19.7	87
	3	» . . . 32	14.4	10.6	16.0	25
	1	» . . . 32	14.4	11 2	14.9	14
	3	Bayrisch spät . . . 9	15.6	9.6	12.5	13
	3	» . . . 1	15.7*	12.3	17.2	76
	?	Braunschweiger . . 18	13.7	10.2	17.3	23
	3	Saaz 130	15.1	9.3	11.9	31
	3	» 2	14.4*	10.0	13.3	12
	3	» 132	14.0	9.1	14.8	42
	3	» 73	13.2	9.8	15.0	20
	3	Saaz roter 75	12.2	11.4	17.1	33
2. English plants.	3	Golding 36	15.0*	11.7	15.5	33
	4	» 36	15.0*	11.2	14.5	17
	4	Brambling 40	15.4	9.6	13.4	31
	3	» 40	15.4	8.9	12.6	19
	?	Fuggles 175	13.6	11.0	15.9	30
	4	» 38	13.1	11.8	13.6	6
	3	Early Hobbs . . . 61	13.2	10.1	14.6	21
	3	Early Bird 167	13.3	7.4	8.3	4
3. Danish plants.	?	Asperup 207	8.2	7.8	10.0	11
	2	» 210	8.7	7.3	7.3	1
	4	Graa 41	8.7	8.8	13.3	35
	3	Grøn 199	9.9	8.4	15.3	26

by crossing with »♂ Nr. 4«, then the proportion between the weights of lupulin produced in the two gardens will, *cæteris paribus*, be as 15.4:9.6. In other words, all that would be obtained by crossing would be a reduction of the quantity of lupulin per unit of surface to $^{96}/_{154}$ or about $^{5}/_{8}$ths of the yield obtained before crossing. A similar result would have been obtained had the experiment been made with one of the other foreign clones mentioned in Table III instead of »Brambling No. 40«. In every case, we find a decrease in the percentage of lupulin.

Such a result as this would seem to afford but poor encouragement to further endeavours in connection with improvement of hops by means of crossing. This is, however, only apparent. A peculiar biological feature in the hop plant here comes to our assistance.

We have hitherto been chiefly occupied with consideration of the average lupulin content in offspring plants. If we now turn back to consider the two first-named specimens: (Table I, the offspring of »Spalter mittel No. 16« and »Bayrisch spät No. 1«, we find that of the 87 and 76 individuals which compose the two sister groups, by far the greater portion are poor, or at least but indifferently good as regards lupulin content. Each group, however, contains some few specimens, the highest plus variates, which are in this respect distinctly excellent.

In Table IV, where the lupulin percentages of the same groups are shown graphically, the position of the mother plant, i. e. its lupulin percentage, is denoted by a horizontal line which divides the group into two parts, the upper containing all those offspring plants with a higher percentage of lupulin than the mother, those beneath the horizontal line being inferior in this respect to the mother plant. In the first case, »Spalter mittel No. 16«, we have thus no fewer than 20 specimens (about 21 %), and in the second 3 specimens (about 4 %) which surpass the mother plant in respect of lupulin content. From Table III, where the maximal lupulin percentages are noted for each offspring group, we see that in most cases, where the number of specimens is fairly large, the maximum value (greatest plus variate) exceeds the value noted for the mother plant.

As a general rule, the number of individuals among the offspring which surpass the mother plant in respect of lupulin

TABLE IV.

Crossing experiments 1911. — Lupulin content of foreign mother plants and offspring, graphical view. Lupulin content of mother plants denoted by horizontal lines.

Lupulin percentage	No. of daughter plants
	»Spalter mittel No. 16« × »♂ No. 3«
20	o
19	
18	o o
17	o o o
16	o
15	o
14	o o o o o o o o o o
	mother plant — 13.2
13	o o o o o o o o o o o o o o o o
12	o o o o o o o o o o o o o o o
11	o o o o o o o o o o o o o o o o
10	o o o o o o o o o o o o o
9	o o o o o o o o
8	o
	»Bayrisch spät No. 1« × »♂ No. 3«
17	o
16	o o
	mother plant — 15.7
15	o o o o o o
14	o o o o o o o o o o o o o o o o
13	o o o o o o o o o o
12	o o o o o o o o o o o
11	o o o o o o o o o o o o
10	o o o o o o o o
9	o o o o o o o
8	o
7	o

content will naturally be but small; this point would, however, for practical purposes, seem less important. The principal thing is, that some few extreme plus variates arise from the crossings, plants having a very high percentage of lupulin. If this feature proves to be maintained under other conditions and in other years, then a clone is produced with distinctly superior lupulin content, and all that is then required will be to propagate the seedling in question by vegetative means. This will, in the

course of some few years, furnish a supply sufficient for the planting of large grounds with a variety giving a high yield of lupulin.

A point of importance in crossing work is, it would seem, the production of a large number of daughter plants. We have here to reckon with the rule, that an increasing number of variates essentially increases the probability of finding exceptionally high plus variates (i. e. individuals with an exceptionally high percentage of lupulin) — and this is exactly what it is desired to obtain. The high lupulin percentages obtained by crossing may then be maintained by vegetative propagation of the extreme plus variates. In this respect, the work of improvement among hops seems more favourably situated than when dealing with many other cultivated plants, which cannot be propagated by vegetative means.

It would seem hopeless to attempt, on the basis of these experiments, any determination as to what has, genetically speaking, taken place in the course of these crossings. Obviously, we may have here to deal with very complicated processes, both in respect of segregations and new combinations. Moreover, we know, at most, only the qualities of the mother plants. As to the male parents, we know nothing of their genotypic character with regard to lupulin content. All that we do know is, that they are wild plants, whose ancestors have probably not been subjected to any kind of artificial »improvement«. This very fact, however, renders it most probable that they are genotypically of a low order as regards lupulin content, which supposition is further supported by the figures found in the crossing experiments. We saw, when comparing mother- and daughter-plants (cf. p. 172 and Table III) that the percentage of lupulin, when considered as an average, decreases in the offspring, which may at any rate be taken as partly due to paternal influence. To obtain a clearer idea as to the influence of the father plant on the lupulin content of the offspring, it will be necessary to supplement the experiments by crossings with a male plant having high lupulin percentage. It may be mentioned that an experiment of this nature is already in progress.

It has been stated in the foregoing, (p. 172) that the average percentage of lupulin decreased in the offspring. If should be added, however, that the rule is not altogether without excep-

tion. We see from Table III that it applies to all the foreign (German, Austrian and English) plants, and that the decrease is often very considerable. Turning, however, to the Danish plants noted in Group 3, we find somewhat different conditions prevailing. The decrease is greatest in »Grön No. 199«, viz: from 9.9 to 8.4: this is, however, only 1.5 %. In »Asperup No. 207« it is still less, being here only 0.4 %, and finally, in the third Danish variety, »Graa No. 41« we find that no decrease has taken place. The average lupulin percentage of the offspring is here the same as in the mother plant, viz: 8.8 as against 8.7. Among the offspring plants themselves, the percentage of lupulin varies from 4.9 to 13.3.

If we now take all the foreign plants as one group, and all the Danish as another, and calculate the mean for all the average percentages of lupulin, we obtain results similar to those found when dealing with the experiments singly. This will be seen from Table V, which gives, as concisely as possible, the essential difference which exists between the foreign and the Danish plants. We see that on crossing, the lupulin percentage falls on an average 3.7 (from 14.1 to 10.4) in the foreign plants, whereas the Danish only show a decrease of 0.8 (from 8.9 to 8.1).

This peculiarity will perhaps be more easily understood if the following facts are borne in mind. The foreign plants all originate from districts, where hop-growing is an important industry, and where the price obtained by the producers is essentially dependent upon the quality of the hops. It is otherwise in Denmark. The hop industry here, which is now practically restricted to the island of Funen, is in a very poor condition, both as regards production and trade[1]). As a matter of fact, the producers obtain the same price per pound for their hops, regardless of what variety these may belong to. It is thu seasy to understand that the producers are far more interested in obtaining the greatest quantity than in any considerations of

[1] Danish hops are used as a rule only for home brewing in the country (especially in Jutland and Funen; to a lesser degree in Sealand). This means that they are employed in the production of a liquor which in respect of quality and uniformity etc. is naturally not expected to reach a very high standard. Under these circumstances, it is not surprising that the standard of quality demanded in the hops themselves should be similarly low.

TABLE V.

Crossing experiments 1911. — Extract of Table III, showing (as averages) the different extent of decrease in lupulin percentage for offspring of foreign and Danish plants.

Mother plants		Offspring		
	Lupulin percentage (average)		Lupulin percentage (mean of all averages)	Decrease
1. Foreign mother plants (20 plants)	14.1 %	1. Offspring of 20 plants in all 566.	10.4 %	3.7 %
2. Danish mother plants (4 plants)	8.9 %	2. Offspring of 4 plants in all 73	8.1 %	0.8 %

quality: in other words, there is no particular reason for producing hops rich in lupulin.

These points should be taken into consideration when discussing the results of our crossing experiments, with the great difference found between the Danish and foreign plants with regard to decrease in the lupulin percentage of the offspring. In the case of the foreign plants, we have to deal with clones selected and cultivated with especial regard to the attainment of a high lupulin content, whereas this consideration would be of greatly subordinate importance in the case of the Danish plants. It is possible, nevertheless, that some of the foreign plants differ in a genotypical sense but slightly from the poorer or intermediate Danish plants as regards lupulin content. and that for this reason the offspring of such plants revert, on an average, more towards a low lupulin content than the offspring of the latter.

Our experiments seem to show. that it is not difficult to produce plants which surpass the mother plant in lupulin content. The lower this value in the mother plant, the easier will it be to raise daughter plants with a higher percentage of lupulin. This is distinctly evident when we compare Table VI p. 180 with Table IV on p. 176. In Table VI, the lupulin percentage in the offspring of the Danish plant »Graa No. 41« is shown graphically, and the percentage of the mother plant (8.7) indicated by a horizontal line in the same manner as in Table IV. It will be noticed that a very large number of the offspring plants (over

TABLE VI.

Crossing experiment 1911. — Lupulin content of Danish mother plant and offspring: graphical view. Lupulin content of mother plant denoted by horizontal line.

Lupulin percentage	No. of daughter plants
	»Graa No. 41« × »♂ No. 4«
14	
13	o
12	
11	o o
10	o o o o o o o o
9	o o o o o o o o o
	Mother plants — 8.7
8	o o o o o o o o o o o o
7	o o o
6	o
5	o
4	

half) here exhibit higher lupulin percentages than the mother, and even among the comparatively few (35) daughter individuals in our experiment, we find one with the high percentage of 13.3. When more than half the daughter plants, however, exhibit higher lupulin percentages than the mother, and when this happens even in cases where wild male plants have been employed in the crossing, it must be admitted that the improvement of the »Graa« hop — the most important Danish variety — in respect of lupulin content would not seem to be a difficult matter.

As regards the foreign varieties, which are richer in lupulin, the results of our crossings show that in many cases, offspring variates have been produced having a higher lupulin percentage than the mother plant. True, these are fewer in number than was the case in the experiments with Danish plants, as will be clearly seen, for instance, on comparing the figures for »Bayrisch spät No. 1« (Table IV) with those for »Graa No. 41« (Table VI); the chances of obtaining high plus variates may, however, be increased by increasing the number of variates: i. e. by sowing a greater number of the seeds obtained by crossing.

Thus the results of the crossing experiments would seem to

be encouraging, more especially considering the fact that in our 1911 experiments, only wild male plants were used, i. e. individuals which would probably be genotypically poor in lupulin. We may, I think, be justified in assuming, that by employing genotypically lupulin-rich male plants, as has been done in subsequent experiments not yet completed, the chances of a good result will be essentially increased. This is also suggested, it seems, in the report by E. S. Salmon above quoted, where reference is made, on p. 30, to a seedling obtained by crossing an English Cantury Whitebine with an American male plant from the Oregon district, where varieties very rich in lupulin are grown, so that the males there may be supposed to be genotypically rich in lupulin. As to this seedling, (Ref. No. K. 22) which showed on analysis 15.24 % lupulin, the writer states[1]): »It is decidedly of interest to find that the use of not only the *female* Oregon hop, but also of the *male* Oregon hop, results in the production of plants with high resins-contents«.

In dealing with the present material, I have been assisted by Frk. cand. polyt. Johanne Bjerrum and Hr. mag. sc. Ö. Winge, by the former in carrying out the many lupulin analyses and by the latter in the arrangement of the experiments and the compilation of the material. I hereby express my best thanks to both for their good work.

IV. Summary.

1. Since 1911, a selection of hop plants belonging to the most important home and foreign varieties have been cultivated in the experimental garden of the Carlsberg Laboratory. Two series of investigations have been made with these plants, both having practical aims: viz:

A. Investigation as to the constancy of valuable qualities in older clones, and

B. Formation of new clones with combinations of valuable qualities.

The qualities which have hitherto been taken in consideration are: a) flowering time, b) productivity, c) lupulin content and d) aroma.

[1]) It should be noted that our lupulin analyses include all resins, soft and hard, whereas those of Mr. Salmon deal with the soft resins only.

2. The present work deals with the investigations as to lupulin content in plants raised by crossing.

The plants used in the crossing consisted of 21 females of Danish, English, German and Austrian origin, all cultivated plants, and wild Danish males. The crossings were carried out in 1911, the seeds sown in the spring of 1912, and the seedlings (744 in number) investigated in the season 1913.

In the lupulin analyses, the total content of soft and hard resins was determined, and is stated as a percentage of the dry weight of the sample in question (»the lupulin percentage«).

3. Graphical comparison of the lupulin percentage in a number of sister plants shows that these arrange themselves in a group, with the majority of individuals placed close to the average value for the total number of individuals (variates) decreasing in number out towards the rarer maximum and minimum values (the extreme plus and minus variates) (cf. Table I, p. 171).

4. In some of the larger crossing experiments we found, despite the fact that wild males, presumably genotypically poor in lupulin, had been used for crossing, that offspring variates having a higher lupulin content than the mother plant were produced (cf. Table III). We have thus produced the necessary conditions for improvement in respect of lupulin content, as those seedlings which are richest in lupulin (the highest plus variates) may be propagated vegetatively, by which means there is a chance that the valuable quality found will be maintained. The work of improvement with hops seems therefore more favourably situated than in the case of many other cultivated plants, where vegetative propagation is impossible.

The number of offspring variates with higher lupulin percentage than the mother plant varied in our experiments according to the richness of the latter in lupulin, and was thus less in the case of the foreign plants rich in lupulin than in the poorer Danish plants, showing for instance about 4 % as against over 50 % (cf. Table IV »Bayrisch spät No. 1« and Table VI »Graa No. 41«). The work of improvement is thus naturally easier when it is desired to raise the lupulin percentage in a poor variety than when dealing with a sort already rich in lupulin. It must be remembered, however, that the finding of but a single very high plus variate may be sufficient, as this

can then be propagated vegetatively. It is moreover possible to increase the chances of obtaining high plus variates by increasing the number of the variates themselves.

5. The relation between the lupulin percentage of the mother plant and the average lupulin percentage of the offspring varies greatly, according as we have to deal with foreign plants rich in lupulin, or poor Danish plants. In the former, the average lupulin percentage of the offspring was essentially lower than that of the mother plant, whereas in the latter case, little or no difference was apparent, (cf. Table III and VI). This is presumably due, at least partly, to the fact that male plants of poor or intermediate lupulin content were used in the crossing experiments.

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JOHS. SCHMIDT:

INVESTIGATION ON HOPS

(*Humulus lupulus*, L.).

VII. THE EMPLOYMENT OF ARTIFICIAL LIGHT IN TITRATION OF THE RESINS IN HOPS.

BY

SVEND HOEG LARSEN.

1. Introductory remarks.

In the course of the work carried out in the Physiological Department of the Carlsberg Laboratory in connection with the quantitative determination of resins in hops[1]), according to the method formulated by Ö. Winge and J. P. H. Jensen, it was found that the darkness of the short winter days — especially in cloudy weather — rendered accurate observation of the transition during the titration a matter of some difficulty. This disadvantage I have endeavoured to obviate by the employment of artificial light, an innovation which will, I trust, further enhance the value of the method in question.

In these experiments, I have employed a titration table of the dimensions stated in the illustration here given.

As will be seen, the table consists of a wooden box, the upper side of which has been removed, and replaced by a plate of ground glass *G*, which is illuminated from below by an electric lamp *L*. The most practical lamp for the purpose, I found, was a metal wire lamp of 25 candle-power, which gives sufficient light without any inconvenience to the eyes. The box is arranged to open sideways by means of hinges, which are shown turned backward in Fig. 2. The lamp can thus by easily

[1]) Investigations on hops (*Humulus lupulus*, L.). IV. A method for quantitative determination of resins in hops, by Ö. Winge and J. P. H. Jensen, (Comptes rendus des travaux du laboratoire de Carlsberg, vol. 11, p. 116, 1914).

removed; it will also be noted that the switch *K* is outside the box. The glass plate is likewise removable, being framed on three sides only, the fourth — that to the left in the drawing — being free. The upright *S* serves as a support for the lamp.

Fig. 1.

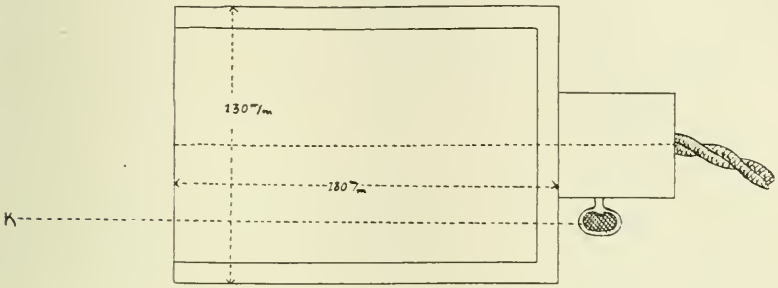
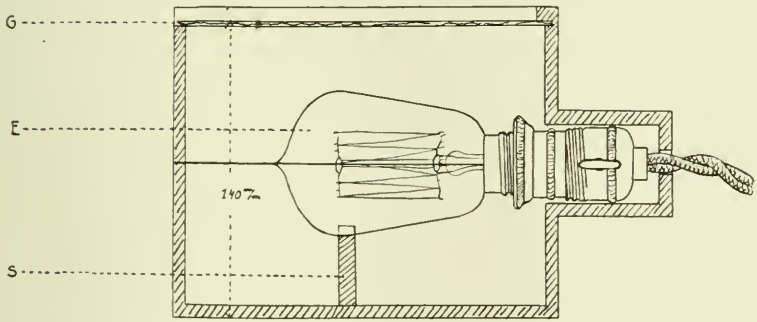


Fig. 2.



Explanation of Fig. 1 and 2.

G Glass plate. *L* Electric lamp. *S* Support. *K* Switch.

2. The experiments.

The principal object of the experiments was to effect a comparison between the results of titrations made according to the present method and those of ordinary titrations carried out in daylight. In addition, other points of difference which may appear on analysis have also been included, such as the influence of the personal equation, and the percentage of accuracy. The results of the investigations were as follows:

1) For purposes of comparison with ordinary titration in daylight, the following analyses may be quoted, each of the results here stated being the mean of two analyses.

TABLE I.

Sample No.	Titration by daylight % lupulin	Titration by artificial light % lupulin
1214	15.5	15.6
1212	15.7	16.3
639	14.5	14.6
194	11.8	11.3
984	10.9	10.5
985	12.3	12.1
1222	14.7	14.8
1219	15.9	15.5
1220	14.7	14.6
1019	9.9	9.9
157	11.6	11.8
841	6.5	6.1

2) In order to ascertain the percentage of accuracy obtainable by the method in question, a series of analyses of the same hop sample have been carried out, as shown in Table II, from which it will be seen that the extreme deviation from the mean amounts, in the one case to 3.9 %, in the other to 2.7 %.

TABLE II.

Sample No.	Results (% lupulin)
116	10.2 10.2 10.1 10.4 10.1 10.7 10.2 10.6
	Average 10.31. Extreme deviation from mean 3.9 %.
52	12.9 12.8 12.4 12.6 12.6 12.7 13.1 12.9
	Average 12.75. Extreme deviation from mean 2.7 %.

3) In order to ascertain whether two analysts could obtain identical results, two investigators took each two samples of three lots of hops: it will be seen from Table III that the extreme deviation is 3 %, the results obtained by the one analyst being sometimes higher, sometimes lower that those of the other.

TABLE III.

Sample No.	Analysed by A % lupulin (mean of 2 analyses)	Analysed by B % lupulin (mean of 2 analyses)
155	12.2	12.0
167	12.4	12.6
55	9.9	10.2

4) All the analyses hitherto mentioned were made with hops crushed in a mincing machine: Table IV gives a comparison of the results obtained from analyses of crushed and whole hops respectively. Two samples of crushed, and two of whole hops were here taken from each variety; the maximum deviation is here 3.3 %.

TABLE IV.

Sample No.	Crushed hops, % lupulin (mean of 2 analyses)	Whole hops, % lupulin (mean of 2 analyses)
420	9.2	9.3
R. 9	12.1	12 5

From the results above quoted it will be seen that this method of titration is by no means inferior in accuracy to titration by daylight, and as the employment of artificial light renders it possible to employ the Winge-Jensen method of investigation in dull weather, or even without daylight at all, I imagine that the apparatus here described will, in many cases — possibly also in other spheres of work — be of some value to the analyst.

3. Summary.

I have in the foregoing described an apparatus for use in titration of lupulin solutions by artificial light, and stated the results of some experiments made with the same, partly for purposes of comparison with ordinary titration by daylight, and also with regard to other features. From these it is evident that the method in question is not inferior to that ordinarily employed as regards accuracy, while possessing, on the other hand, the advantage of being practicable in cases where daylight is insufficient or altogether absent.

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JOHS. SCHMIDT:

INVESTIGATIONS ON HOPS

(*Humulus lupulus*, L.).

VIII. ON THE FLOWERING TIME OF PLANTS RAISED BY CROSSING.

BY

JOHS. SCHMIDT.

1. Introduction.

A point of great practical importance to the hop-grower is the flowering time of the different varieties: in determining how far a certain sort is likely to do well in a given locality, it is always essential to consider whether the variety in question is »early« or »late«. The flowering time has therefore been included among those features which are subjected to regular observation in the hop-growing and crossing experiments carried out at the Carlsberg Laboratory.

As regards the general character and aim of these experiments, I may refer the reader to previous papers in the present series, where other qualities of the hop, viz: the aroma[1]) and the lupulin content[2]) have been dealt with. The following pages will be devoted exclusively to consideration of the flowering time in plants raised by crossing.

In contrast to all other important qualities which are only apparent in the female plants that actually bear the hops, the flowering time is also apparent in the male plants. We have thus, as far as this character is concerned, an opportunity of taking into consideration qualities directly noticeable in the father plant (»earliness«, »lateness«) and allowing these to guide us in selection of the same. In other words, it is probable, or

[1-2]) »Investigations on Hops (Humulus Lupulus L.)«. V. On the aroma of hops, by Johs. Schmidt — and IV. On the lupulin content in plants raised by crossing, by Johs. Schmidt (Comptes-rendus du Laboratorie de Carlsberg, Vol. 11, p. 149—163 and p. 165—183, 1915).

at any rate possible, that we may be able to affect the flowering time of the offspring by paying due regard to that of the male plants when crossing.

It should be remembered that the different clones of male plants, like those of the female, flower at different times: thus I know Danish male plants which flower one after the other from the middle of July to the end of August. In England, where every endeavour is made to induce the production of seeds in the hops, it is naturally of importance in a hop garden to have male plants which flower at the same time as the female; otherwise, nothing would be gained from the presence of male plants on the ground. This point has been particularly investigated, and its importance emphatically pointed out by E. S. Salmon[1] on the basis of investigations carried out at the South-Eastern Agricultural College at Wye, in Kent, England. Growers in Kent and Surrey can now obtain, from the hop gardens of this institution, cuttings of male plants which are known to flower at the times best suited to the needs of each.

The period of time during which the different individual plants are in bloom, i. e. bearing male flowers with loose pollen or female flowers ready for fertilisation, may vary somewhat according to locality and weather conditions, but is, on the whole, of considerable extent. For one male plant (No. 253) grown in our experimental nursery, we have the following particulars, from observations made on alternate days during the months in question (1913): On the 18th July, the first flowers had commenced to open and to shed the pollen. On the 30th July, the plant was in full bloom, with the majority of flowers open, and shedding the pollen. Finally, on the 14th August, we find a note to the effect that the production of pollen is now at an end. In this case, then, the flowering time extended from about 18th July to about 14th August, with a maximum about 1st August. The plant should thus be characterised as flowering about 1st August; at the same time it should be noted that it has exhibited pollen for the greater part of a month. The rule in such cases will probably be that pollen is shed found during a period of from three weeks to a month.

[1]) E. S. Salmon and Arthus Amos: On the value of the male hop — and E. S. Salmon: Notes on Hops 1908—1909, 1912—1913 and 1913—1914 (Journal of S. E. Agricultural College, 1908, 1909, 1914).

In the case of the female plants much the same conditions will be found. Here also we use the expression »flowering begins« at a certain time, this being the date about which the oldest and best lighted inflorescences become ready for fertilisation, while the term »full bloom« denotes that the majority are in that condition. At this time, the fully developed and widely separated styles give the inflorescences an appearance as of small brushes: when the majority of the bines flower simultaneously, the plant will, at some little distance, appear as though draped with a light veil. It is this »full bloom« to which the hop-growers of Funen refer when saying that the hops »purrer«, which again corresponds to the English expression »in burr«[1]). The time at which it appears is naturally to some extent a matter of judgement, but would seem to be rather easily determined. The duration of full bloom however, depends to a great extent upon whether pollination has taken place or not. In the former case, the styles soon fall off, and the »veil« about the plant disappears. Where no pollination takes place, the styles may be retained for weeks, the inflorescences preserving their brush-like appearance, a point which will doubtless have been noticed by anyone having had occasion to prevent pollination by enclosing flowering branches in paper bags.

The flowering time of female plants, which may thus be said to centre about the time of »full bloom«, may also be of considerable extent, as the youngest inflorescences, on branches less favourably lighted than the rest, do not flower until the majority have passed full bloom.

From this it follows, that it is possible in crossing to unite a male and a female plant flowering — or rather reaching full bloom — at widely different times, the last remains of pollen from a very early male, for instance, being available for pollination of the earliest open flowers from a late female. In this way, an attempt might be made to transmit the quality of »earliness« to some of the offspring of a late-flowering female plant, possibly valuable in itself for its high lupulin content.

As regards the time which elapses between the date at which

[1]) The similarity between the word »purre« as used in Funen and the English local term »burr« appears remarkable. I need not, however, here do more than mention the resemblance, leaving the explanation to others more versed in the subject.

a plant is in »full bloom« and that at which is is »ready for picking«, this varies both for the different plants and in different years. Roughly speaking, it should be between 5 and 4 weeks.

2. The experiments.

Detailed particulars as to the manner in which the experiments were carried out will be found in the report previously published, on the lupulin content in plants raised by crossing. A few main facts will here suffice. The crossings were made during the period from end of July to end of August 1911, the mother plants being selected from various Danish and foreign cultivated varieties grown in the experimental garden of the Carlsberg Laboratory. There being no cultivated male plants available in the garden in 1911, pollen was collected from wild hops growing in a wood in North Sealand (Færgelunden at Jægerspris). The pollen was taken, as far as possible, from one plant only (»♂ No. 3«) the bines of which covered the surface of a bush; we cannot, however, be certain that all these bines belonged to the same clone, or that all the seedlings were derived from the same father. ♂ No. 4 was isolated from »♂ No. 3«. As regards flowering time, it may be mentioned that the plant was in full bloom about middle of August, so that the father must probably be called intermediate in this respect. Finally, in the case of a single experiment made in 1911, an earlier-flowering male (♂ No. 1) was used, this plant having been found growing up a fence near the Laboratory. As to its flowering time, the records state that by 31 July 1911 it had already passed the full bloom stage. The flowering shoots of this plant were rooted up and discarded immediately after use.

Promiscuous pollination was guarded against by enclosing the female shoots in paper bags. This process is likewise described at length in the previous paper above referred to[1]).

The seeds obtained from the crossings were sown in the spring of 1912, and a number of the seedlings flowered the same year. All the plants were transferred to their ultimate position during the autumn of 1912. The observations as to flowering time all refer to the season 1913, after which the greater part

[1]) »Investigations on Hops (Humulus Lupulus L.)«. VI. On the Lupulin-content in plants raised by crossing. By Johs. Schmidt (Comptes-rendus du Laboratoire de Carlsberg, vol. 11, p. 169, 1915.

of the seedlings were rooted up owing to want of space, so that we have not been able to extend the observations over several years. Only in the case of the female plants have we sufficiently detailed records as to flowering time to permit of their being dealt with here.

The female plants used in the experiments included both Danish and foreign varieties, the latter imported from hop districts in Germany, Austria and England.

The results of the investigation will be found in Table I. All data noted for the offspring refer to the season 1913; in the case of the mother plants, however, I have further stated the average date of full bloom for the two years 1912 and 1913, reliable observations being here available for both years[1]).

As regards the offspring, the particulars stated are as follows: In each group of sister plants, the date of full bloom in the earliest and latest flowering individuals respectively, and in addition, the average date of full bloom for all sister plants.

By way of example, we may select from the table the data given for the offspring of »Bayrisch spät No. 1« × »♂ No. 3«. We have in this case examined a considerable number — in all 51 — of the daughter plants.

The flowering time »full bloom« for these 51 plants varies considerably, extending from the 16th July to 28th August. Only a few of the plants, however, flower so early or so late as these extreme limits. By far the greater part occupy an intermediate position in this respect, grouping themselves about the 11th August, which is the average date for all the plants in question. Altogether, the dates for flowering time of (»full bloom«) these sister plants present a similar appearance to the figures for lupulin content (l. c. p. 171, Table I) i. e. the majority of individuals (variates) falling close to the average, the numbers then decreasing considerably towards the two extremes (extreme plus and minus variates). The distribution of variates in the offspring is thus in the main identical for both qualities, lupulin content and flowering time.

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[1]) A number of the plants received from England, especially the »Golding«, were found to be so late in flowering, and ripened so poorly, that we soon discontinued the cultivation of these varieties. Among these were some few of these used in the crossing experiments of 1911. The dates of flowering given in the tables for these plants therefore refer only to the year 1912.

We have thus, in the crossing experiment with »Bayrisch spät No. 1« obtained some very early, some intermediate, and some very late plants. In view of the impossibility of subjecting a large number of plants to exactly identical external conditions, it must be presumed that some slight differences in this respect have exerted a certain influence on the date of flowering, and that a rearrangement of the plants would effect a certain alteration in the dates in question. Making allowance for this, however, it may be taken as proved, by these experiments, that there is every possibility of improving a variety in respect of flowering time, as the most suitable variates obtained can always be propagated vegetatively. The acquisition of a hop-clone, otherwise valuable in itself, and with the additional advantage of attaining »full bloom« as early as 18th July — which was the case with the earliest variate in our experiment above mentioned — may be of great importance in the Danish climate. With so early a variety as this, the grower will almost invariably be able to count on having his hops thoroughly ripe, even in the poorest summer, the value of which is obvious. And on the other hand, in countries where climatic conditions allow of a longer period of vegetation, it may again be advantageous to select a late variate, late-flowering plants being frequently superior in point of yield than the earlier varieties.

A comparison of the flowering time in offspring and mother plants presents features of considerable interest. First of all, we may take those cases where the mother plants are either very early (plus variates) or very late (minus variates). To the former class belong most of the plants from Saaz in Bohemia; to the latter, those received from Kent, in England, under the name of »Golding«. Our crossing experiments in 1911 included several plants of both these groups.

In Table II, the figures for a group of early plants from Saaz and a group of late varieties from England (»Fuggles« and »Golding«) are separately noted. In each case, the average date of full bloom in 1913 is given, together with the corresponding date for the offspring, this latter calculated as a mean of the averages for the individual sister groups. In addition, the difference between the average flowering dates of mother plants and offspring has been noted in No. of days.

TABLE I. Crossing experiments 1911. — **Flowe**

Parentage ♂ No.	Parentage ♀	♀ No.	»Full bloom« (♀) 1912—1913 (Average)	»Full bloom« (♀) 1913
3	Saaz	130	29—30. July	23. July
3	»	132	30. July	28. July
3	»	75	4—5. August	4. Augu
3	Saaz, roter	73	7. August	8. Augu
3	Spalter, mittel	16	11. August	6. Augu
3	» »	136	7—8. August	6. Augu
3	» »	32	18—19. August	22. Augu
1	» »	32	18—19. August	22. Augu
3	Bayrisch spät	1	10—11. August	8. Augu
3	» »	9	12. August	8. Augu
?	Braunschweiger	18	9—10. August	11. Augu
3	Early Bird	167	6—7. August	30. July
3	Brambling	64	1912: 13. August	
3	»	40	5. August[1])	4. Augu
3	Early Hobbs	61	12. August	18. Augu
3	Fuggles	175	18—19. August	22. Augu
4	»	38	15. August	22. Augu
3	Golding	36	21. August[2])	23. Augu
?	»	44	1912: 20. August	
?	»	93	1912: 22. August	
3	»	187	1912: 22. August	
3	Asperup	207	3—4. August	4. Augus
3	Grøn	199	6—7. August	4. Augus
4	Graa	41	7. August	6. Augus

[1]) 1912: 6. August.

[2]) 1912: 19. August.

s of mother plants and their offspring.

Offspring (♀ only)				Difference	
»Full bloom« 1913			No. of plants	No. of days between »full bloom« in mother plants and offspring (average)	
erage of daughter plants	Earliest specimen	Latest specimen		Mother plants 1912—1913 (Average)	Mother plants 1913
August	5. August	20. August	44	+12—13	+19
August	30. July	23. August	59	+11	+13
August	30. July	20. August	30	+ 8—9	+ 9
August	7. August	30. August	24	+ 4	+ 5
August	5. August	25. August	90	+ 4	+ 9
August	19. July	20. August	39	+ 1—2	+ 3
August	19. July	22. August	37	÷ 7—8	÷11
August	28. July	20. August	17	÷13—14	÷17
August	16. July	28. August	51	+ 0—1	+ 3
. August	22. July	28. August	23	÷ 4—5	÷ 0—1
August	29. July	20. August	25	÷ 4—5	÷ 6
).August	8. August	14. August	8	+ 3	+10—11
. August	24. July	20. August	27	÷ 5—6	
August	5. August	28. August	51	+ 5	+ 6
August	5. August	14. August	61	÷ 3	÷ 9
August	18. July	20. August	26	÷ 6—7	÷10
August	5. August	20. August	5	÷ 2	÷ 9
August	29. July	28. August	43	÷11	÷13
August	24. July	20. August	20	÷13	
August	29. July	20. August	41	÷15	
August	4. August	28. August	37	÷10	
August	8. August	28. August	11	+ 6—7	+ 6
August	5. August	28. August	71	+ 5—6	+ 8
. August	5. August	20. August	34	+ 1—2	+ 2—3

TABLE II.

Crossing experiments. — Flowering times of mother plants and their offspring compared. Extract of Table I.

Mother plants		Offspring		Difference
	»Full bloom« (average)		»Full bloom« (mean of averages)	
1. Early plants »Saaz« (No. 130, 132, 75, 73) (average of 4 plants)	29. July	1. Offspring of 4 early plants, total 133 individuals	11. August	+ 13 days
2. Late plants »Fuggles« (No. 175, 38) »Golding« (No. 36) (average of 3 plants)	22. August	2. Offspring of 3 late plants, total 74 individuals	12. August	÷ 10 days

It will be seen from the table, that the average flowering dates of the offspring groups differ essentially from those of the mother plants, the early varieties (»Saaz«) producing offspring flowering 13 days later than the mother plant, whereas the offspring of the later English varieties (»Fuggles« and »Golding«) flower 10 days earlier than the mother plants.

The peculiarity here noted applies to plants selected from the earliest and latest specimens; if we compare, however, the figures in Table I for the individual mother plants and their offspring, we find that the rule applies throughout. In every case, the average flowering time is retarded in the offspring of early plants, while the reverse is the case with the offspring of later varieties. On the other hand, the offspring of intermediate mother plants exhibit little or no difference in this respect, the average flowering time of the offspring almost coinciding with that of the mother plant (cf. Table I, e. g. »Bayrisch spät No. 9« and »Graa No. 41«].

That the feature here observed is an actual fact would seem to be beyond doubt, especially when we consider the large number of plants examined, and the great difference in the dates. It should moreover be noted that the difference is apparent in both directions i. e. the flowering being in some cases accelerated, in others retarded.

As to the causes of this phenomenon, nothing can be said with certainly at the moment. It would appear natural, how-

ever, to regard paternal influence as at any rate one possible factor. It will be remembered. that in almost all our crossings, the pollen employed was taken from a intermediate male plant, (No. 3). It would seem reasonable enough to suppose that early female plants crossed with an intermediate male should produce offspring flowering on an average later than the mother, whereas, on the other hand, the offspring of a late female plant crossed with the same intermediate male might well flower earlier than the mother plant[1]). It is doubtful, however, whether this alone can be regarded as sufficient to occasion the very considerable alterations in the flowering time which are apparent in several cases.

In the observations here dealt with, I have been assisted by the Laboratory custodian Mr. N. R. Poulsen, and the Laboratory gardener Mr. Chr. Smith, to whom I wish to express my thanks.

3. Summary.

1. The present work deals with the investigations made in 1911 as to flowering time in plants raised by crossing.

The crossings were made with 23 Danish, English, Austrian and German plants, all cultivated varieties, the males being wild Danish plants. The crossings were made in the summer of 1911. The seeds were sown in the spring of 1912, and the seedlings, 874 in all, examined in the summer of 1913.

2. A comparison of the date of flowering in a number of sister plants shows that they may vary greatly in this respect, and that they group themselves (as in the case of the lupulin content) with the majority of individuals (variates) falling close to the average date of flowering for all. From this average, the numbers decrease greatly towards the two extremes, represented by the earliest flowering specimen (the highest plus variate) and the latest (lowest minus variate) respectively.

[1]) We may in this connection again refer to Table I, from which it will be seen that the same female plant (»Spalter mittel No. 32«) was crossed both with the intermediate »♂ No. 3« and the earlier »♂ No. 1«. The result showed that the offspring of the former crossing flowered on an average later than that of the latter (with the earlier male). This experiment is, however, still too isolated to warrant any definite conclusion.

This renders is possible to improve a variety in respect of flowering time, by selecting the most suitable variates and propagating them vegetatively.

3. A comparison of offspring and mother plants reveals the fact that the mean flowering date of the former is often essentially different from that of the latter. This made itself apparent in our experiments as a retardation of the flowering time in the offspring of early plants, and an advancement of the same in those of later varieties, while in the offspring of intermediate mother plants, little or no difference was observed. The extent of the difference in number of days is noted in the last columns of Table I.

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SUR L'EMPLOI DE SOLUTIONS DE BORAX ET D'ACIDE BORIQUE DANS LA DÉTERMINATION COLORIMÉTRIQUE DE LA CONCENTRATION EN IONS HYDROGÈNE DE L'EAU DE MER

PAR

SVEN PALITZSCH.

La méthode colorimétrique élaborée par S. P. L. Sörensen et Palitzsch et décrite antérieurement dans ces mêmes Comptes-rendus[1]), pour la détermination de la concentration en ions hydrogène de l'eau de mer, repose sur la comparaison des nuances de coloration produites par l'addition d'un réactif indicateur convenable, soit à un échantillon de l'eau en question, soit à une série de liquides dont on connaît déjà la concentration en ions hydrogène. A cet effet, on s'est servi jusqu'ici de mélanges, en proportions convenables, de solutions de borate de sodium et d'acide chlorhydrique ainsi que de phosphates primaire et secondaire. Ces liquides de comparaison ont jusqu'à présent donné entière satisfaction; les difficultés ne surgirent que lorsque M. le Professeur Roald Amundsen exprima le désir de voir appliquer notre méthode dans cette expédition qu'il se propose de consacrer à l'exploration des eaux baignant le pôle nord. La durée de l'expédition étant fixée à quatre ans, il sera indispensable de préparer les principales solutions à bord du navire, et cette préparation ne laissera pas de présenter quelques inconvénients. C'est de la préparation de la solution de borate que naisseront surtout les obstacles à vaincre, cette opération exigeant l'emploi d'une solution d'hydroxyde sodique exempte d'acide carbo-

1) Comptes-rendus du Laboratoire de Carlsberg, **9**, 8 (1910); **10**, 85 (1911); **10**, 252 (1913).

nique et ayant une concentration bien déterminée; en effet, non seulement la préparation d'une telle solution à bord, mais aussi sa conservation présenterait tant d'inconvénients, que le besoin s'est fait sentir d'autres liquides de comparaison mieux appropriés à ces conditions particulières.

Il s'agissait donc de trouver deux ou trois substances qu'on choisirait de préférence solides, susceptibles d'être pesées bien exactement, puis conservées dans des bocaux en verre ordinaires sans subir d'altération, et que l'on n'aurait qu'à faire dissoudre dans un volume d'eau déterminé. Il est bien entendu que ces substances devront en même temps satisfaire aux conditions que doit remplir toute substance étalon, c'est-à-dire que leur préparation à l'état de pureté suffisante doit pouvoir se réaliser sans trop de peine, et aussi que la vérification de leur pureté doit pouvoir se faire assez facilement; enfin — condition particulièrement importante dans le cas qui nous occupe — que les mélanges formés de solutions de ces substances doivent être susceptibles de faire l'office de tampons à des concentrations ioniques embrassant la région considérée, et que ces concentrations doivent pouvoir être mesurées avec exactitude aussi bien par voie électrométrique que par colorimétrie.

Or, il a été constaté que toutes les conditions que je viens d'énumérer sont remplies par l'acide borique et le borate de soude ($Na_2B_4O_7$, $10\,H_2O$), substances qui toutes les deux offrent encore cet avantage qu'on peut s'en procurer à un état de pureté à peu près suffisante.

Pour ce qui est de l'acide borique, son application comme aussi le degré de pureté désirable ont déjà été examinés et décrits par S. P. L. Sörensen[1]), qui en a fait usage pour la préparation des mélanges de borate ci-dessus mentionnés.

Quant au borate de soude, il peut, lui aussi, être aisément préparé à l'état de pureté très satisfaisant, et même de façon à présenter la composition théorique, nonobstant le fait qu'à la température et au degré d'humidité ordinaires il cristallise avec 10 molécules d'eau; ajoutons que le borax à 10 molécules d'eau résiste si bien à l'influence de l'air et acquiert par nouvelle cristallisation si exactement la teneur en eau voulue que, comme l'on sait, il se prête éminemment à l'emploi comme substance étalon

[1]) Compte-rendus du Laboratoire de Carlsberg, **8**, 40 (1909).

dans l'analyse titrimétrique dans tous les cas où il ne sera pas indispensable d'apporter aux mesures une exactitude extrêmement rigoureuse.

Dans le but de rechercher, quelles étaient les préparations commerciales de soude boratée qui pourraient servir à la préparation des liquides de comparaison en question, j'en ai examiné deux fournies par C. A. F. Kahlbaum, à Berlin, à savoir «Borax mit Garantieschein» et «Borax zur Analyse», et, d'autre part, du borax «chimiquement pur» marqué «I», ainsi que des échantillons recristallisés une fois des deux dernières préparations.

50 gr. de borax furent dissous dans 50 cc. d'eau chaude, puis filtrés et refroidis rapidement, en agitant; les cristaux furent essorés à la trompe, puis desséchés à l'air sur du papier-filtre. Rendement environ 45 gr.

Les épreuves relatives à la pureté qualitative ont été effectuées sur des solutions à 5 pour cent, dans lesquelles aucune des cinq préparations susmentionnées ne donna la moindre réaction dénotant la présence d'acide carbonique, d'acide sulfurique ou de calcium. L'épreuve faite au point de vue de la présence du fer (en employant le rhodanure de potassium), ainsi que celle ayant trait aux corps précipitables par l'acide hydrosulfurique, ne produisaient que des teintes à peine perceptibles, rose et brunâtre respectivement. Additionnées d'acide nitrique et de nitrate d'argent, les diverses solutions ont été reconnues garder sensiblement leur limpidité, à l'exception de celle de Borax I, laquelle devint nettement opalescente. — Une légère dose de chlorure est assez indifférente comme effet, pourvu qu'elle soit assez peu importante pour ne point se faire sentir dans le dosage des éléments essentiels constitutifs du borax.

La proportion de base a été déterminée par titration au moyen de l'acide chlorhydrique, en employant le rouge de méthyle comme indicateur. Cette dernière substance, qui subit un changement de couleur beaucoup plus marqué que ne le fait le méthylorange dont on se sert communément, vire en effet à une concentration en ions hydrogène où l'état de dissociation de l'acide borique est assez peu prononcé pour que le résultat de la titration n'en soit pas influencé de façon appréciable.

J'ai effectué la titration sur une solution contenant $^1/_{20}$ mol.-gr. de borax par litre, solution dans laquelle la base sera décinormale, de sorte que 25 cc. d'une solution de borax pure

se trouverait correspondre à 25 cc. d'acide chlorhydrique décime, ce qui était le cas, au millième près, pour quatre des cinq échantillons de borax considérés; seul le Borax I demandait $0^{cc},35$ d'acide chlorhydrique décime en plus. C'est tout juste si un écart de cette grandeur pourra se faire sentir dans la mesure colorimétrique: par exemple, un mélange de 5 cc. de borax M/20 et d'une proportion égale d'acide borique se trouvera entaché d'un écart de $0^{cc},07$ de base décime, ce qui correspond à une modification d'environ 0,02 dans la valeur de p_H^+. Etant donné cependant que le borax I, qui avait été recristallisé une fois, s'est montré avoir précisément la teneur en base calculée, il s'en suit qu'il ne faut pas s'épargner la peine de faire subir à ces préparations une recristallisation préalable.

La teneur en eau peut être déterminée au $^1/_{500}$ près, en portant au rouge, à condition toutefois de commencer le chauffage avec précaution et surtout pas trop vite; autrement, le borax se gonflera et sera projeté hors du creuset avec une violence explosive. Finalement il convient de rougir pendant quelque temps le borax fondu, afin d'éliminer la dernière trace d'eau; car, malgré le fait établi par T. H. Norton et D. M. Roth[1]), que le borax est 16 fois plus volatil que le chlorure de sodium, il n'y a pas lieu de craindre que le résultat de l'analyse soit sensiblement altéré par une chauffe peu prolongée dans un creuset de platine[2]). A la suite d'une incinération d'environ 2 gr. de borax, le poids du résidu des cinq échantillons analysés ne s'écartait que de $^1/_{500}$, au plus, de la théorie.

Dosage de l'acide borique. On sait qu'il n'est pas possible de faire le titrage de l'acide borique en solution aqueuse en employant la phénolphtaleine comme indicateur, parce que cet acide est si faiblement dissocié qu'une solution de son sel sodique aura une réaction fortement alcaline; c'est ainsi que, par exemple, dans une solution M/10 de NaH_2BO_3, la concentration en ions hydrogène ne s'élèvera qu'à 10^{-11} environ, correspondant ainsi à celle d'une solution d'hydroxyde sodique normale au $^1/_{1000}$. Conformément à cet état de choses, un titrage a fait constater qu'une solution d'acide borique M/5 est virée au rouge en présence de la phénolphtaleine dès qu'on y ajoute $^1/_{20}$ à $^1/_{10}$ de

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[1]) Journal of the Americ. Chem. Soc., **19**, 156 (1897).

[2]) Sigmund Waldbott: *Ibid.*, **16**, 414 (1894).

son équivalent en hydroxyde sodique par équivalent d'acide borique, même si ce dernier est compté pour monobasique. Même avec un indicateur produisant un virage à la concentration en ions hydrogène 10^{-11}, la titration ne peut pas être effectuée avec exactitude, parce que, à 10^{-11}, l'addition d'une faible dose d'acide ou de base ne produirait pas une modification suffisante de la concentration ionique.

Par contre, on a pu obtenir de bons résultats en ayant recours à la glycérine. Quant à la question — d'ailleurs peu importante au point de vue de l'analyse — de savoir si ce succès est dû à une modification que la dissociation aurait subie par suite de cette addition, en ce sens que l'hydroxyde sodique et l'acide basique (monobasique) seraient arrivés à être dissociés à un même degré, ou bien si c'est exclusivement une formation de composés de glycérine qui entre en jeu et qui nous permet un titrage exact, elle n'est pas encore tranchée avec certitude.

En vue de la titration de la proportion d'acide borique, on chauffa jusqu'à dissolution complète environ $0^{gr},5$ de borax dans 15 cc. d'eau, en présence d'acide sulfurique en quantité légèrement supérieure à la quantité de base (2 cc. 2-n.). Après refroidissement, l'excès d'acide sulfurique fut titré en retour au moyen d'une lessive de soude normale au $^1/_5$ et en présence de rouge de méthyle (5 gouttes d'une solution de $0^{gr},1$ dans 300 cc. d'alcool à 93 % et 200 cc. d'eau). Le virage produit était si bien prononcé, que le titrage fut aussi exact que le mesurage de la liqueur de titration. Enfin, on ajouta 150 cc. de glycérine (ayant un poids spécifique de 1,23), abondamment de phénolphtaleine (1 cc. d'une solution de $0^{gr},5$ dans 50 cc. d'alcool et 50 cc. d'eau) et, en dernier lieu, de la soude normale au $^1/_5$ (25 cc. environ) jusqu'à apparition de la première teinte rouge; comme liqueur de comparaison on se servait d'une solution aqueuse de glycérine. Le virage, déjà perceptible avec $0^{cc},02$ de soude normale au $^1/_5$, était bien net avec $0^{cc},05$, ce qui équivaut à une précision de $^1/_{500}$, au moins, limite d'erreurs dans laquelle les proportions d'acide borique dans les cinq échantillons de borax ont été trouvées égales aux proportions calculées.

Selon les descriptions qu'on a données de la méthode, le titrage de borax ne peut être regardé comme achevé que lorsque la coloration rouge engendrée par la phénolphtaleine ne disparaît plus à la suite d'une nouvelle addition de glycérine, dont, d'après

les indications données, 30 à 40 cc. suffiront en général pour la titration de $0^{gr},5$ de borax. Cependant, ayant constaté au cours des analyses (dont nous venons de rendre compte) qu'il fallait 150 cc. de glycérine, j'ai cherché à mettre en lumière l'effet produit par la dose de glycérine ajoutée, et dans ce but j'ai effectué une titration en procédant comme indiqué ci-dessus, seulement avec cette différence que la glycérine n'était pas ajoutée en une fois, mais par portions successives de 25 cc. chacune. Après chaque addition, la dose de l'hydroxyde de sodium a été lue, et en même temps on la corrigeait de la dose à ajouter au volume correspondant de glycérine pour faire virer celui-ci, en présence de phénolphtaleine, au rouge juste perceptible (cette dose était de $0^{cc},01$ par 25 cc. de glycérine).

On pesa $0^{gr},4970$ de borax, correspondant à $5 \times 0,4970 \times 4000 : 382,16 = 26^{cc},01$ de lessive de soude n. au $^1/_5$. Voici les résultats numériques de la titration:

Glycérine, en cc.....	0	25	50	75	100
NaOH n. au $^1/_5$ ..	env. 2,5	25,48	25,82	25,94	25,97
Ecart	1 : 1,1	1 : 50	1 : 140	1 : 370	1 : 650

Glycérine, en cc.....	125	150	175	200
NaOH n. au $^1/_5$.....	25,99	26,01	26,02	26,02
Ecart	1 : 1300	0	1 : 2600	1 : 2600

Ces chiffres montrent que sans l'addition de glycérine la titration donne un résultat tout à fait erroné, outre que le virage produit est très indistinct. Au contraire, l'addition de 25 cc. de glycérine donne déjà une valeur passablement exacte de la proportion d'acide borique, l'écart entre la quantité d'hydroxyde de sodium trouvée et celle calculée au moyen du poids du borax ne se montant qu'à $^1/_{50}$. En employant 75 cc. de glycérine, on obtient un écart inférieur à $^1/_{300}$, degré de précision qui est généralement considéré comme satisfaisant dans l'analyse volumétrique; ainsi que les valeurs obtenues nous l'apprennent, on peut pourtant, en doublant la dose de glycérine, arriver à une précision plus grande encore, et c'est pourquoi dans les analyses exposées ci-dessus on en a employé 150 cc. Si dans l'essai considéré l'erreur est nulle pour cette proportion de glycérine et négative pour des doses plus fortes, c'est évidemment un pur hasard, puisque l'erreur totale à laquelle peuvent donner lieu le pesage

et la neutralisation de la glycérine sans addition de borax s'élève facilement à $\frac{1}{2000}$.

En ce qui concerne le dosage de l'acide borique, il est à noter en outre, que l'acide carbonique, s'il y en a, sera compris dans le titrage de l'acide borique, et que, par conséquent, il est indispensable d'expulser l'acide carbonique en faisant bouillir pendant cinq minutes la solution encore sulfurique. A cet effet, il convient de se servir d'un réfrigérant ascendant, afin que l'acide borique ne soit pas entraîné par les vapeurs d'eau; comme la solution de borax sera alors diluée par l'eau servant à rincer le dit appareil, il s'en suit qu'il faudra un peu plus de 150 cc. de glycérine pour obtenir le même degré d'exactitude que précédemment. Une analyse exécutée de cette manière a donné tout à fait le même résultat qu'une autre effectuée sans ébullition, d'où il est légitime de conclure que la préparation examinée ne contenait pas d'acide carbonique et, d'autre part, que, en apportant ce perfectionnement à la méthode, on ne court pas le risque de fausser les résultats.

Ainsi donc, il résulte de l'ensemble de ces analyses que les deux échantillons fournis par Kahlbaum présentaient un degré de pureté suffisant pour permettre de les employer tels quels, et que le «Borax I» n'avait qu'à être soumis à une seule recristallisation pour pouvoir servir. Il est donc permis de dire que la réalisation du borax suffisamment pur a été trouvée, en somme, très facile à obtenir. D'ailleurs, afin de se rendre compte si telle préparation se prête à la préparation de liqueurs de comparaison, il ne sera généralement pas nécessaire de procéder à toutes les déterminations mentionnées dans ce qui précède; d'ordinaire, on pourra se borner à la titration de la quantité de base. —

Ensuite, il s'agissait de choisir les concentrations les plus convenables des solutions principales dont les mélanges variés devaient fournir les liqueurs de comparaison. La condition principale à remplir, c'était de prendre soin qu'une différence déterminée dans la concentration en ions hydrogène — dans la région la plus importante au point de vue des recherches concernant l'eau de mer — correspondît à la différence la plus grande possible dans les proportions des divers ingrédients; en effet, on obtiendrait ainsi qu'un mesurage insuffisamment exact ait le moins d'influence possible sur la concentration ionique des liquides de comparaison.

Pour cela, la concentration en ions hydrogène a été déterminée dans un certain nombre de mélanges de borax M/40 avec de l'acide borique M/5, de borax M/20 avec de l'acide borique M/10, et de borax M/20 avec de l'acide borique M/5. Toutes ces mesures montrèrent clairement que ce dernier mélange était le plus avantageux; aussi en ai-je fait l'objet d'un examen plus approfondi[1]).

Comme une solution de borax se comporte tout à fait de la même manière qu'un mélange formé par les proportions correspondantes d'hydroxyde de sodium et d'acide borique. il s'en suit que la solution de borax M/20 sera identique à la solution de borate utilisée auparavant et qui était constituée par de l'hydroxyde sodique et de l'acide borique de façon à être n. au $^1/_{10}$ relativement à cet hydroxyde et M/5 relativement à l'acide borique; toutefois, elle est notablement plus facile à préparer.

Comme on le voit, dans des mélanges de borax M/20 et d'acide borique M/5 en proportions différentes, la concentration de cet acide sera invariablement M/5, tandis que celle de l'hydroxyde sodique — et, par conséquent, celle des ions hydrogène — se trouvera sujette à des variations. En conséquence, l'erreur de sel sera sous la dépendance de la concentration des ions hydrogène, contrairement à ce qui était le cas pour les mélanges boratiques primitifs. Comme nous l'avons montré plus haut, l'erreur de sel est due à la modification provoquée par les sels dans la coloration de l'indicateur, indépendamment de l'influence qu'ils exercent sur la concentration ionique; quant à la grandeur de cette erreur, elle est égale à la différence entre l'exposant des ions hydrogène trouvé par la méthode colorimétrique et celui établi par électrométrie.

A titre d'exemple indicatif de la grandeur variable de l'erreur de sel se rapportant aux solutions de borax et d'acide borique, nous citerons ce résultat que pour un mélange de 1 cc. de borax M/20 et 9 cc. d'acide borique M/5 elle s'est trouvée être de 0,1 plus grande que le borax M/20. Afin de remédier à l'inconvénient auquel cela donnerait lieu lors de l'emploi des liquides de comparaison, la solution d'acide borique fut additionnée d'une faible dose de chlorure de sodium; une addition

[1]) En outre. j'ai réalisé des mesures de phosphate primaire M/15, mélangé de borax soit M/20, soit M/40; mais la concentration ionique aussi bien que l'erreur de sel de ces mélanges se trouvèrent subir des modifications si irrégulières, qu'on ne pouvait pas les utiliser.

de ce corps telle que la solution d'acide borique devint décimoléculaire par rapport à lui, et qu'on tenta d'abord parce que la teneur en sodium serait alors la même dans tous les mélanges, se trouva cependant être trop forte, car elle eut pour effet de rendre l'erreur de sel plus petite de 0,1 qu'elle ne l'était pour le borax seul; par contre, le chlorure sodique n. au $^1/_{20}$ donna une erreur de sel qui dans les limites d'erreurs de la méthode était exactement la même pour tous les mélanges.

La concentration en ions hydrogène fut maintenant mesurée par la voie électrométrique, à la température de 18°, dans un certain nombre de liquides préparés en mélangeant des quantités convenables de la solution contenant $^1/_{20}$ gr.-mol. de borax pur et de la solution contenant par litre $^1/_5$ gr.-mol. d'acide borique et $^1/_{20}$ gr.-mol. de chlorure de sodium. Les exposants d'ions hydrogène — p_H^+ — ainsi que les concentrations ioniques qui y correspondent, se trouvent réunis dans le tableau ci-contre. Ces données, on le sait, permettent de calculer facilement l'exposant des ions hydroxyle en déduisant p_H^+ de 14,14, et la concentration en ions hydroxyle comme l'antilogarithme de cet exposant avec signe négatif. Les valeurs de p_H^+ ont été soit calculées comme les moyennes des résultats numériques de plusieurs mesures, ou bien obtenues moyennant un ajustement graphique, qui cependant n'a en aucun cas été supérieur à 1 dans la deuxième décimale de p_H^+.

Vu la légère incurvation que présente sur la plus grande partie de son parcours la courbe résumant la dépendance qui existe entre l'exposant d'ions hydrogène et les proportions relatives des constituants du mélange, on est à même de calculer par simple interpolation les valeurs intermédiaires, ce qui n'était guère possible pour les solutions boratiques utilisées antérieurement; ce n'est que pour les mélanges plus acides que celui formé de 2cc,5 de borax M/20 et de 7cc,5 d'acide borique M/5, qu'on est obligé de tracer la courbe sur du papier quadrillé au millimètre et de faire des interpolations graphiques.

Lorsqu'il s'agit de recherches d'ordre hydrographique, recherches auxquelles ces solutions sont destinées tout particulièrement, on a le plus souvent besoin de mélanges contenant 6,0 — 5,5 — 5,0 — 4,5 — 4,0 — 3,5 — 3,0 — 2,3 — 1,5 — de borax M/20; ce n'est que pour les eaux d'une alcalinité particulièrement faible (renfermant de l'acide hydrosulfurique) que ceux à 2,5 — 2,0 —

Concentration en ions hydrogène des mélanges de borax et d'acide borique.

Borax M/20 cc.	Acide borique M/5 cc.	P_H^+	$C_H^+ \times 10^9$
10,0	0,0	9,24	0,58
9,0	1,0	9,11	0,78
8,0	2,0	8,98	1,05
7,0	3,0	8,84	1,45
6,0	4,0	8,69	2,04
5,5	4,5	8,60	2,51
5,0	5,0	8,51	3,09
4,5	5,5	8,41	3,89
4,0	6,0	8,31	4,90
3,5	6,5	8,20	6,31
3,0	7,0	8,08	8,32
2,5	7,5	7,94	11,5
2,3	7,7	7,88	13,2
2,0	8,0	7,78	16,6
1,5	8,5	7,60	25,1
1,0	9,0	7,36	43,7
0,6	9,4	7,09	64,6
0,3	9,7	6,77	170

1,5 — 1,0 — 0,6 — 0,3 M/20 de borax pourront aussi être utiles; on n'a pas jusqu'ici rencontré d'eau marine plus acide ou plus alcaline.

En ce qui concerne l'erreur de sel de ces nouveaux mélanges de borates, elle n'a pas été déterminée par des mesures directes de l'eau. Vu, en effet, que de telles mesures ont été effectuées très soigneusement pour les solutions boratiques primitives, il paraît pleinement suffisant d'en mesurer les nouvelles par la méthode colorimétrique, en employant celles-là comme liqueurs étalons; si alors on arrive au même exposant d'ions hydrogène que celui trouvé par électrométrie, les erreurs de sel sont de même grandeur pour les deux solutions de borax. C'est ce que j'ai pu constater en employant soit la phénolphtaléine soit la naphtolphtaléine, et l'emploi du rouge neutre a donné le

même résultat (dans ce dernier cas, j'ai utilisé comme liquides de comparaison des mélanges de phosphates).

On voit donc que l'erreur de sel s'exprime par les mêmes chiffres que ceux antérieurement établis par S. P. L. Sörensen et Palitzsch[1]) pour toutes les concentrations salines se présentant dans l'eau de mer.

Il appert des courbes représentant ces déterminations, que pour les trois indicateurs susmentionnés les erreurs de sel sont les suivantes:

	35 ‰ de sel	20 ‰ de sel	10 ‰ de sel
Phénolphtaleine.....	÷ 0,21	÷ 0,16	÷ 0,09
Naphtolphtaleine.....	÷ 0,22	÷ 0,17	÷ 0,09
Rouge neutre	+ 0,10	+ 0,05	+ 0,00

(La phénolphtaléine est propre à servir pour les mélanges contenant des proportions de borax M/20, allant de 10 cc. à $3^{cc},5$, la naphtolphtaléine pour ceux dont ces proportions sont comprises entre 4,5 et 0,6, enfin le rouge neutre pour ceux où elles n'atteignent que 2 cc. à $0^{cc},3$).

Les solutions principales sont préparées et conservées de la manière ordinaire, en tenant dûment compte de l'acide carbonique de l'air[2]); pour la préparation de la solution de borax, on dissout $19^{gr},108$ de borax, et pour la solution d'acide borique $12^{gr},404$ de cet acide avec $2^{gr},925$ de chlorure de sodium dans de l'eau bouillie, puis on complète jusqu'à un litre. Lorsqu'il s'agit d'une expédition lointaine, on pèse avant le départ les quantités voulues des substances solides dans des bocaux de verre bouchés à l'émeri et sur lesquels on attache du papier parchemin.

Le fait que, au cours d'une longue expédition sur mer, on éprouve souvent des difficultés à se procurer de l'eau bouillie, m'a conduit à examiner jusqu'à quel point il est rigoureusement nécessaire de faire bouillir l'eau A cet effet, j'ai fait passer, pendant environ 4 heures, de l'air atmosphérique par l'eau distillée destinée à servir de dissolvant; une comparaison faite par colorimétrie des liqueurs ainsi préparées avec d'autres analogues, pour lesquelles j'avais employé de l'eau bouillie, n'a décelé aucune différence (comme indicateurs j'ai employé tant la phénol-

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[1]) Comptes-rendus du Laboratoire de Carlsberg. **10**, 252 (1913), et **9**, 19 (1910).
[2]) *Ibid.*, **10**, 95 (1911).

phtaléine que la naphtolphtaléine). Ainsi, dans le cas considéré, une faible proportion d'acide carbonique semble être inoffensive. — Il va sans dire que, au contraire, la solution de borax alcaline ne doit point être exposée à l'action de l'air.

Il est vrai que ces nouveaux mélanges de borates sont principalement destinés aux mesurages hydrographiques; cependant, vu la facilité et la promptitude avec lesquelles on peut les préparer — on n'a qu'à dissoudre dans de l'eau une dose pesée de la substance en question, pour compléter ensuite cette solution jusqu'à un volume déterminé —, on pourra en faire usage partout où l'on aura besoin de solutions dont l'exposant des ions hydrogène soit situé entre 7 et 9; d'ailleurs, les mélanges de borax M/20 et d'hydroxyde sodique n. au $^1/_{10}$ seront de tous points identiques aux mélanges de ces substances indiqués par S. P. L. Sörensen[1]).

Les mélanges de borax seront, par exemple, tout indiqués pour servir de solutions de contrôle dans la titration au formol; car pour cet usage il sera évidemment inutile de préparer d'abord les deux solutions principales pour en faire ensuite le mélange: on n'aura qu'à dissoudre toutes à la fois dans de l'eau bouillie les proportions de borax, d'acide borique et de chlorure sodique calculées en vue de la concentration ionique voulue.

En procédant de la manière très commode que nous venons d'indiquer, on peut préparer aussi le mélange de borax utilisé par P. A. Kober et K. Sugiura dans leur méthode de détermination microchimique des acides aminés[2]). La solution utilisée par ces savants ayant $p_H^+ = 8{,}8$, on voit que le mélange à employer sera constitué par $6^{cc},7$ de borax + $3^{cc},3$ d'acide borique additionnés de NaCl. 1 litre d'une telle solution contiendra donc

$0{,}33 \times 12{,}404 = 4^{gr},093$ d'acide borique,
$0{,}33 \times 2{,}925 = 0^{gr},965$ NaCl et
$0{,}67 \times 19{,}108 = 12^{gr},802$ de borax.

Ces quantités sont un peu supérieures à celles qu'on trouve en prenant comme point de départ les mélanges de borate et d'acide chlorhydrique employés par P. A. Kober et K. Sugiura (3 borate + 1 acide chlorhydrique d'après Sörensen), ce qui donnerait:

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[1]) Comptes-rendus du Laboratoire de Carlsberg, **8**, 43 (1909).
[2]) Journal of the Amer. Chem. Soc., **35**, 1558 (1913).

$0{,}25 \times 12{,}404 = 3^{gr},101$ d'acide borique,
$0{,}25 \times 5{,}846 = 1^{gr},462$ NaCl et
$(0{,}75 - 0{,}25) \times 19{,}108 = 9^{gr},554$ de borax dans 1 litre de solution.

Pour terminer, il faut remarquer que, dans un mémoire paru en 1908 et dont je n'ai eu connaissance qu'après avoir fini la présente étude, C. L. A. Schmidt et C. P. Finger[1]) ont préconisé l'emploi du borax et de l'acide borique pour la préparation de liqueurs de comparaison, cependant sans qu'ils aient vérifié si ces substances s'y prêtent.

Je tiens à renouveler encore ici à M. le Professeur et Dr S. P. L. Sörensen mes remerciements dévoués pour le précieux concours qu'il m'a prodigué pendant le cours de ces recherches.

[1]) The Journal of Physical Chemistry, **12**, 406 (1908).

Avril 1915.

ETUDES SUR LA SYNTHESE DES ACIDES AMINES.

X. ACIDE ALLYLHIPPURIQUE.

PAR

S. L. P. SØRENSEN.

DANS un mémoire publié il y a dix ans[1]), j'ai décrit une méthode de préparation, d'un côté, de l'éther allyle-phtalimidomalonique dont la constitution est:

$$(COOC_2H_5)_2C(C_3H_5)\cdot N\begin{matrix}CO\\CO\end{matrix}C_6H_4$$

et, d'autre part, de la glycocolle allylique:

$$CH_2:CH\cdot CH_2\,CH(NH_2)\cdot COOH,$$

indiquant en même temps que ces composés seraient probablement propres à servir de matières premières pour la préparation de l'acide α-amino-γ-δ-dioxy-n-valérique:

$$CH_2(OH)\cdot CH(OH)\cdot CH_2\cdot CH(NH_2)\cdot COOH.$$

Cependant, en poursuivant ces recherches, j'ai reconnu que, comme matière première de ces synthèses, il serait avantageux d'utiliser l'acide allylhippurique:

$$CH_2:CH\cdot CH_2\cdot CH(NH\cdot CO\cdot C_6H_5)\cdot COOH,$$

substance qu'on peut facilement préparer à partir de l'éther allyle-phtalimidomalonique et dont, dans une communication préliminaire parue en 1908[2]), j'ai brièvement décrit le mode de préparation et les propriétés, en signalant que cet acide se prête éminemment à servir comme point de départ dans la préparation synthétique non seulement de l'acide dioxyaminovalérique mentionné ci-dessus, mais encore de plusieurs autres acides α-amino-n-valériques disubstitués.

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[1]) Comptes-rendus des travaux du Laboratoire de Carlsberg, **6**, 186, 1905.

[2]) Ber. der d. chem. Gesellschaft, **41**, 3387 (1908).

Depuis cette époque, j'ai été empêché par d'autres occupations de reprendre ces études, et c'est pourquoi une description détaillée de la préparation et des propriétés de l'acide allylhippurique n'a pas été publiée jusqu'à présent. Tout récemment, cependant, au cours de deux stages semestriels faits dans notre Laboratoire de Carlsberg, M. E. Hammarsten, de Stockholm, a réalisé quelques synthèses à partir de l'acide allylhippurique, travaux dont il rendra compte dans le mémoire suivant. Il paraît donc opportun de donner d'ores et déjà une description complète de la préparation et des propriétés des divers composés qui ont servi de point de départ aux recherches de M. Hammarsten; c'est pour cette raison que je juge à propos de faire paraître à présent le court exposé qu'on va lire.

L'éther allyle-phtalimidomalonique est préparé par le procédé souvent décrit dans des mémoires antérieurs, c'est à dire par chauffage de l'éther phtalimidosodomalonique en présence de l'iodure allylique:

$$(COOC_2H_5)_2CNa\cdot N\begin{matrix}CO\\CO\end{matrix}C_6H_4 + C_3H_5J$$

$$= (COOC_2H_5)_2C(C_3H_5)\cdot N\begin{matrix}CO\\CO\end{matrix}C_6H_4 + NaJ$$

(Pour les détails, nous renvoyons à la partie expérimentale du présent exposé).

Le produit huileux de la réaction ne pouvait pas d'abord être amené à cristalliser[1]); mais, l'éther allyle-phtalimidomalonique une fois obtenu sous forme cristalline, il a depuis été relativement facile, par une recristallisation convenable dans l'alcool étendu, d'obtenir ce composé à l'état nettement cristallin et parfaitement pur, avec un rendement atteignant à peu près 80 pour cent du calcul.

En saponifiant l'éther allyle-phtalimidomalonique par chauffage avec de la lessive de soude:

$$(COOC_2H_5)_2C(C_3H_5)\cdot N\begin{matrix}CO\\CO\end{matrix}C_6H_4 + 3\,NaOH$$

$$= (COONa)_2C(C_3H_5)\cdot NH\cdot CO\,C_6H_4\cdot COONa + 2\,C_2H_5\cdot OH,$$

[1]) Ces mêmes Comptes-rendus, **6**, 187 (1905).

puis décomposant l'acide phtalamique-allyle-malonique tribasique formé, par évaporation en présence de l'acide chlorhydrique:

$$(COOH)_2\,C\,(C_3\,H_5)\cdot NH\cdot CO\cdot C_6\,H_4\cdot COOH + H_2\,O$$
$$= COOH\;\;CH\,(C_3\,H_5)\cdot NH_2 + CO_2 + C_6\,H_4\,(COOH)_2,$$

on peut éliminer le sel marin et la majeure partie de l'acide phtalique en traitant le résidu par l'acide chlorhydrique à 33 pour cent, et le reste de l'acide phtalique peut être extrait à l'éther. La solution chlorhydrique de glycocolle allylique ainsi obtenue sera, après évaporation, puis élimination de l'acide chlorhydrique — ce qui se fait le plus aisément à l'aide du carbonate d'argent — propre à servir à la préparation de la glycocolle allylique, dont les propriétés et l'analyse ont été l'objet d'un mémoire précédent[1]), qui contient en même temps la description du sel cuivrique correspondant peu soluble:

$$(CH_2 : CH\cdot CH_2\cdot CH\,(NH_2)\cdot COO)_2\,Cu.$$

La solution chlorhydrique de la glycocolle allylique peut cependant aussi servir à la préparation directe de l'acide allylhippurique:

$$CH_2 : CH\cdot CH_2\cdot CH\,(NH\cdot CO\cdot C_6\,H_5)\cdot COOH,$$

à savoir par benzoylation en milieu alcalin; après purification convenable et, finalement, recristallisation dans le benzène, l'acide allylhippurique peut s'obtenir à l'état de pureté, avec un rendement d'environ 85 pour cent de la quantité calculée à partir de l'éther malonique substitué mis en œuvre.

Dissous dans le chloroforme, l'acide allylhippurique fixe à la température ambiante, facilement et quantitativement, deux atomes de brome, avec formation de l'acide α-benzoylamino-γ-δ-dibrome-n-valérique:

$$CH_2\,Br\cdot CHBr\cdot CH_2\cdot CH\,(NH\text{-}CO\cdot C_6\,H_5)\cdot COOH,$$

composé qui — ainsi qu'on devait s'y attendre — perd facilement du bromure d'hydrogène, avec formation d'une lactone bromée:

$$CH_2\,Br\cdot \underbrace{CH\cdot CH_2\cdot CH\,(NH\cdot CO\cdot C_6\,H_5)\cdot CO}_{O}$$

Ce sont ces deux substances que M. Hammarsten a examinées de plus près et utilisées comme matières premières dans

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[1]) Ces mêmes Comptes-rendus, **6**, 186 (1905).

les expériences relatées dans le mémoire qui fait suite à celui-ci.

A ce propos, il est à remarquer que l'acide allylhippurique ne renferme qu'un seul atome de carbone asymétrique (en position α); ce n'est qu'à la suite de l'addition de brome qu'il s'en forme encore un (position γ). Il me vint dès lors à l'esprit de chercher, avant la bromuration, à scinder l'acide allylhippurique synthétiquement préparé en ses deux formes optiquement actives, pour essayer ensuite — après la bromuration ou à un stade ultérieur de l'opération — d'une nouvelle scission, correspondant à l'atome asymétrique produit par la bromuration. En ce qui concerne la première de ces scissions, il est possible de la réaliser grâce à ce fait, que le sel de brucine de l'acide allylhippurique lévogyre aussi bien que le sel de chinidine de l'acide dextrogyre sont assez difficilement solubles. — Ayant à préparer ces sels sous la forme cristalline, je me suis heurté au début à des difficultés considérables, qui cependant ont été surmontées plus tard, en ce sens que par amorçage je pus obtenir facilement d'assez fortes quantités de ces corps. Comme cependant les essais de scission, pour lesquels M. J. A. Christiansen m'a prêté un précieux concours, présentaient d'autres difficultés, il faudra reprendre cette question plus tard, quand mes autres travaux me le permettront. Il sera alors à propos de répéter quelques-unes des synthèses effectuées par M. Hammarsten, en partant toutefois des acides allylhippuriques optiquement actifs. J'aime à croire que pour ces recherches futures je pourrai tirer grand parti des résultats déjà acquis grâce aux essais accomplis par M. Hammarsten sur les corps racémiques plus facilement accessibles.

Partie expérimentale.

1. Ether allyle-phtalimidomalonique.

$$(COOC_2H_5)_2C(C_3H_5)\cdot N\begin{matrix}CO\\CO\end{matrix}C_6H_4.$$

Dans un ballon de la capacité d'un litre muni d'un réfrigérant ascendant surmonté d'un tube en U contenant du chlorure de calcium, on chauffa $^1/_2$ molécule-gramme d'éther phtalimidosodomalonique[1]) en présence d'un excès d'iodure allylique fraî-

[1]) Ces mêmes Comptes-rendus, **6**, 10 (1902).

chement distillé, sec (il en a fallu 70 centimètres cubes = environ 129 grammes; calculé: 84 grammes). La température du bain d'huile monta brusquement à 100°, et ensuite, dans le courant d'une heure, à 135°. Le réaction était maintenant en bon train; pour l'accélerer, on agita à plusieurs reprises. Après quatre heures de chauffage à une température comprise entre 130° et 140°, la totalité de l'éther phtalimidosodomalonique avait été transformée, après quoi l'excès d'iodure allylique fut distillé à la vapeur d'eau. Après refroidissement à l'eau glacée, puis décantation de la solution aqueuse brune d'iodure de sodium. on lava une seconde fois au bain-marie bouillant, avec 1/2 litre d'eau, l'huile brunâtre qui était restée. Enfin, après réfrigération à la glace, on décanta l'eau de lavage.

Deux portions — en tout, une molécule-gramme — de l'éther allyle-phtadimidomalonique brut ainsi obtenu, furent alors dissoutes ensemble dans 500 centimètres cubes d'alcool à 93 pour cent. Après filtration de la solution, on lava les ballons et le filtre à 2 fois 80 centimètres cubes d'alcool à 93 pour cent. A l'ensemble de la liqueur filtrée et de l'alcool de lavage on ajouta 200 centimètres cubes d'eau; on constata qu'après cette addition la liqueur demeurait limpide. Ensuite on amorça, et après des agitations répétées la cristallisation commença au bout d'une demi-heure. Le lendemain on essora à la trompe, puis lava les cristaux à 3 fois 150 centimètres cubes d'alcool à 60 pour cent, après quoi on les enleva du filtre pour les triturer dans une capsule avec 300 centimètres cubes d'alcool à 60 pour cent. Finalement, on essora de nouveau et lava avec 150 centimètres cubes d'alcool à 60 pour cent.

L'éther allyle-phtalimidomalonique ainsi obtenu (A) pesait, après dessiccation à l'air, 210gr,5 et était presque parfaitement pur (Point de fusion 61°,5; teneur en azote: 4,04 pour cent; le produit ne renfermait pas d'iode).

Comme, après une addition ultérieure d'eau, le filtratum et l'alcool de lavage, même amorçés, ne firent tomber qu'une huile brunâtre, on distilla l'alcool dans le vide, pour reprendre ensuite dans l'éther l'huile résiduelle. Par dessiccation de la solution éthérée à l'aide de chlorure de calcium, puis distillation de l'éther, on récupéra une huile brunâtre, qui pesait 136 grammes et qui, bouillie avec 1 + 1 + 1/2 + 1/2 litres d'éther de pétrole, laissa un résidu brun d'un poids de 21 grammes. La distilla-

tion de l'éther de pétrole laissa une huile jaune, qui ne tarda pas à cristalliser et qui au bout de deux jours s'était entièrement solidifiée. Ce produit se montra également être constitué par de l'éther allyle-phtalimidomalonique presque pur, abstraction faite d'une faible proportion d'iode, dont il fut débarrassé par dissolution dans 200 + 20 + 20 centimètres cubes d'alcool à 80 pour cent, puis réfrigération, amorçage et repos jusqu'au jour suivant. On put dès lors observer une formation abondante de cristaux, dont la quantité augmenta par l'addition de 40 centimètres cubes d'eau, qui fut réitérée le jour d'après. — Le produit obtenu (B), lavé à 100 centimètres cubes d'alcool à 60 pour cent, pesait 58 grammes, et, en ajoutant encore 80 centimètres cubes, puis 60 centimètres cubes d'eau, on put obtenir un nouveau produit (C), dont le poids était de 8 grammes. Les deux produits B et C étaient exempts d'iode et pratiquement purs (B accusait un point de fusion de 62° et une teneur en azote de 4,01 pour cent; C, point de fusion 61°, teneur en azote 4,01 pour cent).

Le rendement s'élevait donc, au total, à 276gr,5 (environ 80 pour cent de la théorie), et de la dernière eau-mère on put, en la traitant par l'éther de pétrole, récupérer encore une faible quantité du composé pratiquement pur.

Afin d'opérer une purification complète, on fit recristalliser dans de l'alcool à 93 pour cent, qui se prête bien à la nouvelle cristallisation de l'éther allyle-phtalimidomalonique une fois obtenu passablement pur.

200 grammes de ce produit furent dissous au bain-marie dans 200 + 20 + 20 + 20 centimètres cubes d'alcool à 93 pour cent; après refroidissement de la solution vers 30°, on amorça et, en agitant plusieurs fois, on vit bientôt l'éther allyle-phtalimidomalonique tomber sous forme de gros cristaux qui, observés sous le microscope, se présentaient comme des prismes épais, bien développés, à quatre ou six pans; beaucoup d'entre eux étaient surmontés de pyramides de formes diverses, de sorte que les cristaux offraient en partie un aspect assez hétérogène. Le lendemain on essora à la trompe, puis lava les cristaux avec 100 + 50 + 50 centimètres cubes d'alcool à 93 pour cent. Le rendement était de 145gr,8; mais le reste put être recouvré en traitant l'eau-mère par le procédé décrit plus haut.

Dans le dosage de l'azote suivant la méthode de Kjeldahl,

0gr,2523 se trouva correspondre à 10cc,22 d'une solution d'hyposulfite N/14,01 (4,05 pour cent d'azote).

Soumis à la combustion, 0gr,2740 donna 0gr,1335 d'eau (5,45 pour cent d'hydrogène) et 0gr,6287 d'acide carbonique (62,58 pour cent de carbone).

		Calculé	Trouvé
C_{18}	216,00	62,58	62,58
H_{19}	19,15	5,55	5,45
N	14,01	4,06	4,05
O_6	96,00	27,81	
	345,16	100,00	

L'éther allyle-phtalimidomalonique fond sur le «bloc Maquenne» à la température de 61°,5—62°. Il est très soluble, même à froid, dans l'éther, l'acétone, l'éther acétique et le benzène; il est facilement soluble, surtout à chaud, dans les alcools méthylique et éthylique, moins soluble dans l'alcool dilué. L'éther de pétrole le dissout assez facilement, particulièrement à chaud; dans l'eau chaude il est très difficilement soluble, dans l'eau froide pas du tout.

2. Acide allylhippurique.

$$CH_2 : CH \cdot CH_2 \cdot CH\,(NH \cdot CO \cdot C_6H_5) \cdot COOH.$$

On fit dissoudre 1/2 molécule-gramme (172gr,6) d'éther allyle-phtalimidomalonique dans 200 centimètres cubes d'alcool à 93 pour cent renfermés dans une capsule de 4 litres placée au bain-marie. A la solution limpide encore chaude on ajouta, en remuant bien, 400 centimètres cubes d'une lessive de soude 5-normale (calculé 300 centimètres cubes), ce qui ne fit pas précipiter cet éther. Au bout de quelques minutes, la réaction commença à se manifester: on vit se produire un précipité cristallin blanc (probablement le sel sodique de l'acide allyle-phtalamique-malonique), et bientôt le tout formait une bouillie épaisse, qu'on rendit mobile en y ajoutant un peu d'eau. Après une demi-heure de chauffage au bain-marie, la réaction était terminée. L'addition d'un litre d'eau fit alors entrer le tout en dissolution Ensuite on chassa presque tout l'alcool en évaporant au bain-marie jusqu'à la consistance d'une bouillie. En ajoutant 1l,5 d'eau, on eut une liqueur limpide, à laquelle, après quelque refroidissement, on ajouta en remuant 400 centimètres cubes d'acide chlorhydri-

que concentré, qui ne provoqua d'abord que le dégagement d'un peu d'acide carbonique. Au bout de deux ou trois minutes, on vit cependant se former un précipité volumineux blanc (probablement constitué par de l'acide allyle-phtalamique-malonique). Sans y avoir égard, on plaça la capsule au bain d'eau bouillante, ce qui, dans le courant de quelques heures, fit entrer le précipité en dissolution, avec dégagement d'acide carbonique et séparation d'acide phtalique, dont l'aspect macrocristallin permettait de le distinguer aisément d'avec l'acide blanc volumineux mentionné plus haut. Après avoir seringué dans le liquide la couche de sel qui s'était attachée aux parois de la capsule, on fit évaporer, en remuant bien, au volume de $^1/_4$ litre environ, puis, après refroidissement, on ajouta 1 litre d'acide chlorhydrique concentré. La matière fut triturée soigneusement avec un pilon, puis refroidie pendant deux ou trois heures dans l'eau glacée, et filtrée à travers du papier durci[1]). Finalement, on lava le dépôt à 4 fois $^1/_4$ litre d'acide chlorhydrique à 33 pour cent.

Le dépôt, qui renfermait la majeure partie du sel marin et de l'acide phtalique, se trouvait contenir, en tout, 38 mgr. d'azote (environ $^1/_2$ pour cent de la totalité de l'azote). Un lavage à l'eau froide enleva le sel marin et laissa l'acide phtalique, pesant $78^{gr},3$ (calculé 83 grammes).

La solution chlorhydrique fut rapprochée au volume de $^1/_2$ litre, puis épuisée au moyen de 5 fois 1 litre d'éther, et chaque portion d'éther fut lavée à 2 fois 50 centimètres cubes d'eau.

Par distillation de l'éther on obtint $1^{gr},3$ d'acide phtalique, renfermant en tout $2^{mgr},4$ d'azote.

Par chauffage dans un matras capace placé au bain-marie muni d'une toile de sûreté, la solution épuisée à l'éther fut débarrassée de l'éther qui s'y était dissous. Après évaporation au bain-marie jusqu'à consistance sirupeuse, puis dissolution dans de l'eau, on neutralisa au moyen d'une solution 5-normale d'hydroxyde sodique, pour benzoyler ensuite en réfrigérant à la glace par le procédé habituel[2]), en employant du chlorure de benzoyle en proportion $1^1/_2$ fois aussi grande que la quantité calculée, et la proportion y correspondante d'hydroxyde sodique, ajoutées en dix portions: en tout, 105 grammes de chlorure benzoylique et 300 centimètres cubes de lessive de soude 5-normale. La ben-

[1]) Ces mêmes Comptes-rendus, **6**, 52 (1902).

[2]) Ibidem, 45.

zoylation terminée, on ajouta encore 30 centimètres cubes de lessive, et continua d'agiter pendant une heure. Après abandon jusqu'au lendemain, on sépara par filtration un léger dépôt résineux, qu'on lava avec de l'eau contenant de l'hydroxyde sodique.

La solution, réfrigérée à la glace, fut versée dans une ampoule à robinet, puis additionnée d'un excès d'acide chlorhydrique (en tout, 300 centimètres cubes d'acide concentré) et — sans tenir compte du précipité ainsi produit — de $^1/_2$ litre d'éther. Par agitation, on fit dissoudre dans l'éther l'ensemble du précipité (formé des acides benzoïque et allylhippurique), et, après avoir soutiré la couche inférieure aqueuse, on lava avec deux fois 25 centimètres cubes d'eau. La solution chlorhydrique fut extraite encore, de la même manière, à 3 fois 300 centimètres cubes d'éther, chaque portion de celui-ci étant lavée avec 2 fois 25 centimètres cubes d'eau.

Après ce traitement, la solution chlorhydrique contenait, en tout, 167 mgr. d'azote (2,4 pour cent de la totalité de l'azote).

Les extraits éthériques réunis furent desséchés au moyen de chlorure de calcium, après quoi on distilla l'éther; vers la fin de cette opération, on fit passer de l'air à travers le liquide, tout en le chauffant au bain-marie et agitant énergiquement le ballon. L'huile jaunâtre laissée pour résidu et qui était constituée par un mélange d'acide benzoïque et d'acide allylhippurique, fut versée encore chaude dans un mortier, où on la remua bien avec le pilon de façon à prévenir que, en se solidifiant, elle ne formât une masse compacte. Ce qui restait dans le matras fut dissous dans un peu d'éther, et, cette solution ayant été évaporée dans une capsule de porcelaine, le résidu fut ajouté à la portion principale dans le mortier. Réduite en poudre, aussi fine que cela était possible, la masse fut remise dans le matras, où on la fit bouillir durant une heure avec un litre d'éther de pétrole; après décantation de la solution d'acide benzoïque ainsi obtenue, le reste indissous fut de nouveau pulvérisé dans le mortier. On fit bouillir encore avec un litre d'éther de pétrole, et on répéta le même traitement. Après avoir fait bouillir quatre fois avec un litre d'éther de pétrole, on avait obtenu l'acide allylhippurique à l'état presque parfaitement pur (poids après dessiccation à l'air: 102 grammes; calculé $109^{gr},6$).

L'acide benzoïque laissé par distillation de l'éther de pétrole pesait 34 grammes et contenait, en tout, 279 mgr. d'azote (environ 4 pour cent de la totalité d'azote).

En vue d'une purification complète, on fit recristalliser l'acide allylhippurique dans le benzène. Une dissolution de 80 grammes d'acide brut dans 300 centimètres cubes de benzène chaud fut filtrée au moyen d'un entonnoir à filtrations chaudes préalablement chauffé; le filtre subit quatre lavages, chaque fois avec 25 centimètres cubes de benzène chaud. Déjà au cours de la filtration, on vit commencer la cristallisation. La filtration terminée, on fit redissoudre le tout, pour le faire refroidir ensuite, en remuant. On constata bientôt la formation d'un dépôt cristallin lamellé, d'une blancheur éclatante et qui, au microscope, se montra formé de feuillets très minces, bien développés, rhomboïdaux, à peu près rectangulaires, souvent tronqués aux deux coins de façon à présenter la forme d'un hexagone.

Le lendemain on essora à la trompe, puis lava le dépôt à 3 fois 50 centimètres cubes de benzène; ensuite, après un essorage soigneux, on lava deux fois à l'éther, pour éliminer le benzène. Le rendement était de $54^{gr},7$; mais de différentes eaux-mères réunies, on put tirer un supplément d'acide allylhippurique, de sorte que la quantité totale de matière recristallisée pure s'élevait à environ 85 pour cent du calcul.

La substance séchée à l'air, puis abandonnée dans le vide sulfurique, ne perdit pas de son poids.

Dans le dosage de l'azote d'après Kjeldahl, $0^{gr},2110$ se trouva correspondre à $13^{cc},49$ d'une solution d'hyposulfite N/14,01 (6,39 pour cent d'azote).

$0^{gr},2371$ donna par combustion $0^{gr},1250$ d'eau (5,90 pour cent d'hydrogène) et $0^{gr},5727$ d'acide carbonique (65,88 pour cent de carbone).

		Calculé	Trouvé
C_{12}	144,00	65,72	65,88
H_{13}	13,10	5,98	5,90
N	14,01	6,39	6,39
O_3	48,00	21,91	
	219,11	100,00	

L'acide allylhippurique fond au «bloc Maquenne» à 107^0—$107^0,5$. Cet acide est facilement soluble même à froid, dans

les alcools méthylique et éthylique, dans l'acétone, l'éther acétique et le chloroforme; il se dissout facilement dans le benzène à chaud, assez difficilement à froid. L'éther et l'eau froide ne le dissolvent qu'avec difficulté (dans l'eau chaude il se dissout un peu plus facilement).

Dans l'éther de pétrole il est à peine soluble.

Juillet 1915.

ETUDES SUR LA SYNTHESE DES ACIDES AMINES.

XI. 1. ACIDE α-AMINO-γ-δ-DIOXYVALERIQUE.
2. γ-OXYPROLINE.
3. ACIDE α-δ-DIAMINO-γ-OXYVALERIQUE.

PAR

EINAR HAMMARSTEN.

Au cours d'un stage que je viens de faire dans le Laboratoire de Carlsberg, j'ai, à l'instigation de M. le Professeur S. P. L. Sørensen, entrepris des essais de préparer, par la voie des synthèses, des acides α-amino-n-valériques substitués en γ et en δ.

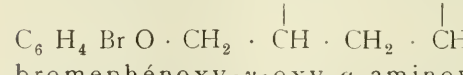
On sait que, même en employant les meilleures méthodes connues jusqu'ici pour l'isolation des acides aminés, on réussit rarement à faire monter la quantité totale des différents produits de décomposition au-dessus de 70 ou, tout au plus, 80 pour cent de celle calculée au moyen de la teneur en azote de l'albumine mise en œuvre. S'il est vrai que le déficit auquel on arrive toujours est attribuable en majeure partie, sinon exclusivement, à la perte de quelques-uns des acides aminés déjà connus comme produits de scission de l'albumine, il n'en est pas moins certain que dans certains cas des combinaisons nouvelles peuvent entrer dans ces pertes. D'autre part, la préparation synthétique de nouveaux acides aminés présente un certain intérêt parce que des produits de transformation de ceux déjà connus figurent probablement comme termes de passage dans les processus d'assimilation et de désassimilation des êtres vivants. Les synthèses de ces produits de transformation possibles ne manqueront pas de faciliter notablement l'étude de leur apparition éventuelle dans le corps des êtres organisés.

Comme un tel acide aminé on peut citer l'acide α-amino-δ-oxy-n-valérique, qui fut préparé, il y a quelques années, par S. P. L. Sørensen[1]). Une autre substance qu'on pourrait regarder en quelque sorte comme un terme intermédiaire entre les acides aminés et les pentoses, serait l'acide α-amino-γ-δ-dioxy-

[1]) Comptes-rendus des travaux du Laboratoire de Carlsberg, **6**, 137 (1905).

n-valérique: $CH_2(OH) \cdot CH(OH) \cdot CH_2 \cdot CH(NH_2) \cdot COOH$, qu'on n'a pas trouvé parmi les produits de scission de l'albumine. En opérant la désamidation du groupe aminé et y faisant entrer un groupe oxhydrile, puis en réalisant la réduction du groupement carboxyle en groupement aldéhyde, on aurait, semble-t-il, une substance qui ne se distinguerait d'une pentose que par l'absence du groupe oxhydrile en position β. D'autre part, si, à l'instar de la plupart des chimistes qui se sont occupés de ces questions, on admet que, au cours de l'assimilation, des acides aminés puissent se convertir en sucre, j'estime que, comme la plus proche substance mère de l'acide dioxy-monoaminé ci-dessus indiqué, il faudrait envisager l'acide α-δ-diamino-γ-oxy-n-valérique ou γ-oxyornithine: $CH_2(NH_2) \cdot CH(OH) \cdot CH_2 \cdot CH(NH)_2 \cdot COOH$, acide aminé qu'on n'a pas non plus, que je sache, isolé parmi les produits de décomposition de l'albumine. Aussi ne serait-il guère à propos, je pense, de discuter jusqu'à quel point il est vraisemblable que l'oxyornithine, ou bien plutôt l'oxyarginine, fait partie intégrante de l'albumine. Il en est de même, et à plus forte raison, de l'hypothèse d'après laquelle une oxyornithine hypothétique pourrait se convertir dans l'organisme en acide α-amino-γ-δ-dioxyvalérique, combinaison également hypothétique Toujours est-il que, pour les raisons que nous venons d'indiquer, la préparation de ces deux acides aminés ainsi que la mise en évidence des propriétés qui les caractérisent, présenteraient bien de l'intérêt.

En ce qui concerne l'acide aminé mentionné en dernier lieu, la préparation en a été tentée par MM. E. Fischer et A. Krämer, qui ont publié sur ce sujet un mémoire intitulé: »Versuche zur Darstellung der α amino-γ-δ-dioxyvaleriansäure«[1]). Partant de l'épichlorhydrine comme matière première, ces savants arrivent en dernier lieu à une substance à laquelle ils assignent la formule de constitution suivante:



$C_6H_4BrO \cdot CH_2 \cdot CH \cdot CH_2 \cdot CH \cdot NH_2$. Ce composé: δ-bromephénoxy-γ-oxy-α-aminovalérolactone, ayant été soumis à la scission, leur fournit, d'un côté, de l'oxyproline et, de l'autre, de faibles quantités d'un corps qu'ils considèrent, à juste titre sans doute, comme étant l'acide aminé cherché. Ils s'expriment là-dessus en ces termes: «..... hat sowohl in freiem

[1]) Berichte der deutschen chemischen Gesellschaft, **41**, 2728 (1908).

Zustande wie nach der Analyse des Kupfersalzes die Zusammensetzung $C_5 H_{11} O_4 N$ und kann demnach die gesuchte α-Amino-γ-δ-dioxyvaleriansäure sein. Leider ist die Ausbeute so schlecht, dass wir bisher nicht in der Lage waren, die Säure gründlich zu untersuchen und ihre Struktur sicher festzustellen».

Pour ma part, je suis parvenu à préparer par synthèse, des combinaisons des acides α-amino-γ-δ-dioxy-n-valérique et α-δ-diamino-γ-oxy-n-valérique. En outre, il sera rendu compte, dans le présent travail, d'une nouvelle méthode de préparation de la γ-oxyproline. — Il est à remarquer qu'il ne s'agit exclusivement que des formes racémiques.

Comme matière première j'ai eu recours à l'acide allylhippurique, dont la préparation et les propriétés ont été décrites par M. S. P. L. Sørensen dans le mémoire précédent.

Cette substance:

$$CH_2 : CH \cdot CH_2 \cdot CH (NH \cdot CO \cdot C_6 H_5) \cdot COOH,$$

est susceptible de fixer facilement deux atomes de brome en position γ-δ, en rompant la double liaison. Par des essais antérieurs de bromuration, S. P. L. Sørensen avait été conduit à la préparation de la combination dibromée à l'état d'une huile, et d'autre part, à une δ-brome-γ-oxylactone de l'acide propylhippurique (Il obtenait cette lactone sous forme cristalline, et seulement en faible quantité). De plus, il avait fait des essais préliminaires en vue de la synthèse du dioxyacide dont il est question plus haut.

En exécutant des essais ayant pour but d'arriver à isoler la combinaison dibromée produite par la bromuration de l'acide allylhippurique, j'ai constaté que la dite combinaison était très prompte à se changer partiellement en la lactone bromée en δ mentionnée ci-dessus, avec séparation de bromure d'hydrogène; le reste de la combinaison aura probablement subi d'autres transformations. J'ai réussi pourtant à isoler sous forme cristalline le dérivé γ-δ-dibromé de l'acide allylhippurique, quoique avec un rendement n'atteignant que 10 à 15 pour cent de la valeur calculée.

L'introduction d'éthyle dans le groupe carboxyle se trouva diminuer la tendance à former de la lactone. C'est pourquoi je procédai à la préparation de l'éther éthylallylhippurique:

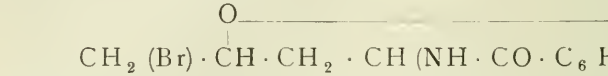
$$CH_2 : CH \cdot CH_2 \cdot CH (NH \cdot CO \cdot C_6 H_5) \cdot COO C_2 H_5,$$

en traitant par du chlorure d'hydrogène desséché une solution alcoolique d'acide allylhippurique. L'éther éthylique formé (environ 90 pour cent du calcul) cristallise bien et se laisse bromurer quantitativement en solution chloroformique. Le dérivé dibromé:

$$CH_2\,(Br)\cdot CH\,(Br)\cdot CH_2\cdot CH\,(NH\cdot CO\cdot C_6H_5)\cdot COOC_2H_5,$$

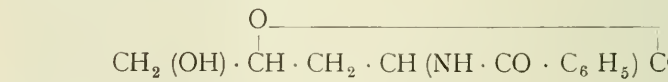
put être isolé sous la forme de cristaux (avec un rendement d'environ 70 pour cent de la valeur calculée sur l'éther).

L'*α*-benzoylamino-*δ*-brome-*γ*-oxyvalérolactone, dont la constitution est:



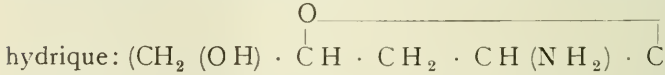
$$CH_2\,(Br)\cdot CH\cdot CH_2\cdot CH\,(NH\cdot CO\cdot C_6H_5)\cdot CO,$$

fut préparée en partant du dérivé dibromé brut de l'acide allylhippurique par traitement de l'eau de baryte saturée à froid en quantité correspondant à l'un des deux atomes de brome. Il est probable que Ba entre dans le groupe carboxyle, pour réagir ensuite, avec formation de lactone, sur l'atome de brome en *γ*. La *δ*-bromelactone, qui se présentait sous forme de cristaux magnifiques, ne put pourtant s'obtenir qu'avec un rendement d'environ 30 pour cent de la quantité théorique. En traitant par un grand excès d'eau de baryte la lessive-mère provenant de la bromelactone ainsi que cette lactone elle-même, on parvint à isoler une substance qui n'est autre que l'acide *α*-benzoylamino-*γ*-*δ*-dioxyvalérique; car portée à l'ébullition en présence d'acide sulfurique 0,2-normal, elle se change en *α*-benzoylamino-*γ*-*δ*-dioxyvalérolactone:



$$CH_2\,(OH)\cdot CH\cdot CH_2\cdot CH\,(NH\cdot CO\cdot C_6H_5)\,CO,$$

qu'on peut facilement amener à former des cristaux, et qui, traitée par l'acide chlorhydrique fort, donna la dioxylactone chlor-



hydrique: $(CH_2\,(OH)\cdot CH\cdot CH_2\cdot CH\,(NH_2)\cdot CO)\cdot HCl$, dépourvue de benzoyle et aussi bien cristallisée que la précédente.

Après élimination de l'acide chlorhydrique au moyen de carbonate d'argent, suivie d'une ébullition avec le carbonate de cuivre, elle fournit le sel cuivrique, d'une belle couleur bleu clair, de l'acide *α*-amino-*γ*-*δ*-dioxyvalérique:

$$(CH_2\,(OH)\cdot CH\,(OH)\cdot CH_2\cdot CH\,(NH_2)\cdot COO)_2\,Cu.$$

Par ébullition de l'acide «dibrome-allylhippurique», dissous dans de l'alcool étendu, avec du carbonate de baryum en excès, on put amener les deux atomes de brome à réagir avec le baryum, réaction qui, contrairement à ce qu'on pouvait attendre, ne donna naissance ni au dioxyacide précédemment mentionné ni à sa lactone, mais bien à un composé qui se trouva être la γ-oxybenzoyleproline:

$$\begin{array}{l} CH_2 — CH \cdot OOC \cdot C_6H_5 \\ | \qquad\quad | \\ CH \qquad CH_2 \\ | \qquad\quad | \\ COOH \quad NH \end{array}$$

En ce qui concerne la manière dont cette réaction s'est passée, il paraît vraisemblable qu'il se forme un anneau avec liaison α-δ — formation à laquelle il y a tendance, ainsi que S. P. L. Sørensen[1]) l'a démontré —, en même temps que l'atome de brome s'unit directement au groupe benzoyle et que, vu la réaction faiblement alcaline, le bromure de benzoyle ainsi formé donne lieu à une benzoylation du groupe γ-oxy.

En effet, pour expliquer d'une manière simple et naturelle la migration inattendue du groupe benzoyle dans la molécule, il me semble que la marche de la réaction peut être exprimée brièvement par le schème ci-après:

I.

$$\begin{array}{l} CH_2\,(Br) \\ | \\ CH\,(Br) \\ | \\ 2.\ CH_2 \\ | \\ CH(NH \cdot CO \cdot C_6H_5) \\ | \\ COOH \end{array}$$

donne avec le carbonate de baryum le sel barytique de:

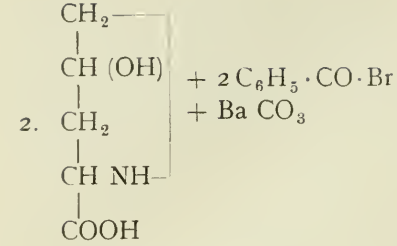
II.

$$\begin{array}{l} CH_2\,(Br) \\ | \\ CH\,(OH) \\ | \\ 2.\ CH_2 \\ | \\ CH(NH \cdot CO \cdot C_6H_5) \\ | \\ COOH \end{array}$$

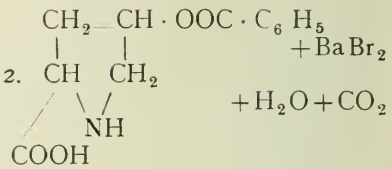
et l'atome de brome δ réagit avec le groupe benzoyle α

[1]) Ces mêmes Comptes rendus, **6**, 145 (1905).

III.



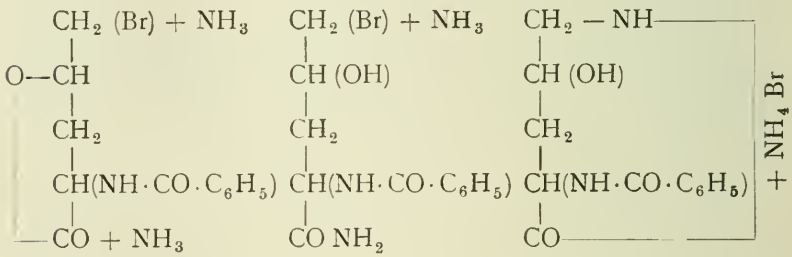
IV.



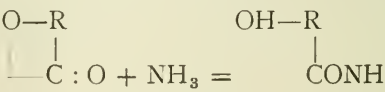
Un chauffage en présence de l'eau de baryte saturée à froid fit séparer le groupe benzoyle, et, après avoir éliminé l'acide benzoïque, on put isoler les deux sels cuivriques de la γ-oxyproline qui, à leur tour, fournirent l'acide aminé lui-même.

La synthèse de la γ-oxyornithine se réalisa à partir de l'α-benzoylamino-δ-brome-γ-oxylactone pure. Le traitement par une solution alcoolique 5-normale d'ammoniaque fit changer la bromelactone en une pipéridone substituée:

I. II. III.



Les deux premières phases de cette réaction (I et II), conformément à la manière dont un éther-sel et l'ammoniaque alcoolique réagissent généralement l'un sur l'autre:



conduisirent à la formation d'amide et d'alcool.

La pipéridone substituée soumise à l'action de l'eau de baryte, entra facilement en dissolution et donna naissance à l'α-monobenzoyle-γ-oxyornithine:

$$CH_2\,(NH_2)\cdot CH\,(OH)\cdot CH_2\cdot CH\,(NH\cdot CO\cdot C_6\,H_5)\cdot COOH.$$

Par ébullition de ce composé en présence d'acide chlorhydrique concentré, on arriva à en éliminer l'acide benzoïque, et à

en isoler divers sels, tels qu'un picrate, un sel double de chlorure de platine, etc.

Il est probable qu'au composé monobenzoylé précité on peut additionner de la cyanamide. Par analogie avec l'addition de cyanamide à la α-monobenzoyle-ornithine, réalisée par S. P. L. Sörensen et Margrethe Höyrup[1]), il en résulterait vraisemblablement l'α-monobenzoyle-γ-oxyornithine:

$$NH : C \cdot NH \cdot CH_2 \cdot CH(OH) \cdot CH_2 \cdot CH(NH \cdot CO \cdot C_6H_5) \cdot COOH.$$
$$NH_2.$$

J'ai déjà institué des essais dans ce sens.

Partie expérimentale.

Ether éthyl-allylhippurique.

$$CH_2 : CH \cdot CH_2 \cdot CH(NH \cdot CO \cdot C_6H_5) \cdot COO \cdot C_2H_5.$$

Dans un matras muni d'un réfrigérant à reflux surmonté d'un tube à chlorure de calcium, on fit dissoudre $^1/_{20}$ molécule (11 gr.) d'acide allylhippurique dans 300 centimètres cubes d'alcool fraîchement distillé sur du sodium; puis on y fit passer, pendant dix minutes, un courant rapide de chlorure d'hydrogène sec. La solution presque incolore, qui contenait maintenant environ 8 pour cent de chlorure d'hydrogène, calculé d'après l'augmentation de poids qu'elle avait subie, fut placée sur le bain d'eau bouillante. Au bout de quatre minutes, le liquide entra en ébullition, laquelle on fit durer une minute, et qui ne détermina pas de coloration foncée appréciable. On fit séjourner alors le matras pendant une heure dans l'eau glacée, puis on neutralisa la solution, prudemment et en refroidissant, au moyen d'alcoolate sodique demi-normal réfrigéré à la glace. Le sel marin ainsi mis en liberté fut essoré à la trompe, et la liqueur filtrée fut rapprochée dans le vide (20 m/m de pression) au bain-marie légèrement chauffé. On fit dissoudre dans 150 centimètres cubes d'éther le résidu, qui était constitué par une matière huileuse d'une couleur jaune grisâtre clair, et on rinça le matras avec 3 fois 50 centimètres cubes d'éther.

Le traitement par l'éther laissa un faible résidu A, et la solution éthérée, desséchée au chlorure de calcium, abandonna un

[1]) Ces mêmes Comptes-rendus, **10**, 121 (1911).

petit précipité B. A se trouva contenir $5^{mgr},5$ et B $11^{mgr},5$ d'azote, dosé d'après Kjeldahl; on les a donc jetés.

Evaporée dans le vide à la température ambiante, la solution éthérée laissa une masse solide blanche cristallisée, dont le poids était de 12 grammes. Après avoir été réduite en poudre dans un mortier, cette masse fut bouillie à reflux avec 5 fois 200 centimètres cubes d'éther de pétrole, chaque fois pendant une demi-heure. La dernière ébullition laissa une faible quantité de matière visqueuse colorée en jaune et formée en partie probablement de matière première ayant échappé à l'éthérification. L'acide allylhippurique, en effet, est à peine soluble dans l'éther de pétrole, tandis que son éther-sel éthylique s'y dissout facilement à chaud. Les extraits d'éther de pétrole réunis soumis au refroidissement, laissèrent tomber pas mal de cristaux d'un blanc pur, dont l'éther de pétrole fut décanté, pour être ensuite évaporé jusque vers 200 centimètres cubes, ce qui détermina une seconde cristallisation, qu'on réunit à la première. Le poids total était de $11^{gr},2$, soit 91 pour cent de la valeur calculée. Une partie des cristaux blanc pur furent soumis directement à l'analyse.

Dans le dosage de l'azote d'après Kjeldahl, $0^{gr},3170$ correspondait à $18^{cc},05$ d'une solution d'hyposulfite norm. au 1/14,01, tandis que $0^{gr},2434$ répondait à 14 cc. de cette solution.

La méthode de Wys pour la détermination de l'indice d'iode dans les graisses donna: $0^{gr},1714$—98,8; $0^{gr},1710$—99,0.

	Calculé p. c.	Trouvé p. c.	
N.....	5,67	5,57	5,62
Indice d'iode	102,8	98,8	99,0

La substance fond sur le «bloc Maquenne» en cinq secondes à la température de $54^{0},5$. L'éther-sel se dissout aisément à la température ambiante, dans l'éther, le chloroforme, le benzène, le toluène; assez difficilement dans l'éther de pétrole à chaud; il est insoluble dans l'eau froide, un peu soluble dans l'eau chaude (avec réaction neutre). Bromuré dans le sein du chloroforme, il fixe facilement 2 atomes de brome. Dans l'éther de pétrole, l'éther-sel cristallise en longues aiguilles, souvent macroscopiques, de forme nettement prismatique.

Ether étyl-α-benzoylamino-γ-δ-dibrome-n-valérique.

$CH_2\,(Br)\cdot CH\,(Br)\cdot CH_2\cdot CH\,(NH\cdot CO\cdot C_6\,H_5)\,COOC_2\,H_5$.

A une dissolution de $^1/_{15}$ molécule (16gr,5) d'éther allylhippurique dans 50 cc. de chloroforme, on ajouta du brome goutte par goutte. On vit les gouttes se décolorer instantanément dans le chloroformé, qui se chauffa légèrement peu à peu. Sans refroidir, on continua la bromuration jusqu'à ce que la dose pesée d'avance eût été ajoutée. Vers la fin, la décoloration se produisit lentement, et finalement la solution chloroformique limpide avait pris une teinte légèrement rouge jaunâtre. Après avoir versé la solution dans une capsule en verre à fond plat, on accéléra la vaporisation du chloroforme en soufflant fortement sur sa surface au moyen d'un ventilateur électrique. Le résidu était formé d'une huile tenace incolore, qu'on ne put pas directement amener à cristalliser et que, après dissolution dans de l'alcool (50 + 10 + 10 cc.), on transvasa dans un gobelet, où l'on y ajouta de l'eau jusqu'à formation d'un petit louche. Le gobelet fut alors placé dans la glacière. Au bout de quelques jours, on sépara par filtration un certain nombre de cristaux qui, lavés avec 3 fois 5 cc. d'alcool à 50 pour cent, puis séchés à l'air, accusèrent un poids de 15 gr. Après évaporation dans le vide de la liqueur filtrée et de l'alcool de lavage, l'huile laissée pour résidu fut reprise dans 15 cc. d'alcool, puis additionnée d'eau jusqu'à ce que le liquide devînt louche. On obtint 1gr,5 de cristaux que, après lavage avec 3 fois 5 cc. d'alcool à 50 pour cent, on réunit à ceux obtenus précédemment. Rendement: 61 pour cent du calcul. — En vue de l'analyse, on recristallisa deux fois, par le procédé ci-dessus décrit, une partie (2 gr.) de la préparation dans l'alcool et l'eau.

D'après Carius, 0gr,1329 donna 0gr,1238 AgBr.

Dans le dosage d'azote suivant Kjeldahl, 0gr,2982 correspondait à 9cc,9 d'hyposulfite N/14,01, et 0gr,3513 également à 11cc,7.

	Calculé	Trouvé
Br.....	39,29	39,66
N	3,44	3,32—3,33.

Fond sur «bloc Maquenne» en 5 secondes entre 96°,5 et 97°,5.

L'éther dibromé se dissout aisément, à la température ordinaire, dans l'éther, l'alcool, le benzène et le toluène; très difficilement dans l'eau, même à chaud. La solution aqueuse présente

une réaction faiblement acide au tournesol, et, par refroidissement, elle fait tomber une huile qui, triturée, se prend peu à peu en une masse cristalline compacte. La solution de ces cristaux dans l'alcool étendu accuse une réaction neutre; séchés à l'air, ils fondent à 97°,5. L'éther dibromé semble donc résister assez bien à l'influence de l'eau. Il cristallise en aiguilles rectangulaires.

Acide α-benzoylamino-γ-δ-dibrome-n-valérique »Acide dibromallylhippurique«.

$$CH_2Br \cdot CHBr \cdot CH_2 \cdot CH\,(NH \cdot CO \cdot C_6\,H_5)\,COOH.$$

La bromuration de l'acide allylhippurique fut effectuée de la même manière que dans le cas de son éther (voir plus haut). Le résidu tenace jaune laissé après la vaporisation du chloroforme fut dissous, à la température ordinaire, dans une quantité d'alcool aussi petite que possible, puis additionné d'éther jusqu'à ce que le liquide commençât de louchir nettement. Au bout de quelques jours, pendant lesquels on vit tomber d'abord un peu d'huile qui par trituration se prit en masse cristalline, puis directement des cristaux, on essora à la trompe, lava à l'éther alcoolique, et fit recristalliser deux fois dans l'alcool en y ajoutant de l'éther en excès. Le rendement etait très faible (environ 10 à 15 pour cent de la théorie).

0gr,1337 donna d'après Carius 0gr,1323 AgBr.

Dans le dosage de l'azote d'après Kjeldahl, 0gr,1256 correspondait à 4cc,40 d'hyposulfite N/14,01.

	Calculé	Trouvé
Br.....	42,19	42,05
N.....	3,7	3,5

Fond au «bloc Maquenne» (5 secondes) à 203—204°, en se décomposant.

L'«acide dibromallylhippurique» est facilement soluble dans l'alcool, insoluble dans l'éther.

α-benzoylamino-δ-brome-γoxyvalérolactone.

$$CH_2(Br) \cdot \underset{\displaystyle O}{\underset{|}{CH}} \cdot CH_2 \cdot CH\,(NH \cdot CO \cdot C_6\,H_5) \cdot \underset{|}{CO}.$$

Après bromuration de 1/4 molécule (environ 55 gr. d'acide allylhippurique) au sein du chloroforme suivant le procédé décrit plus haut, on chassa le chloroforme dans le vide (20 mm., bain-

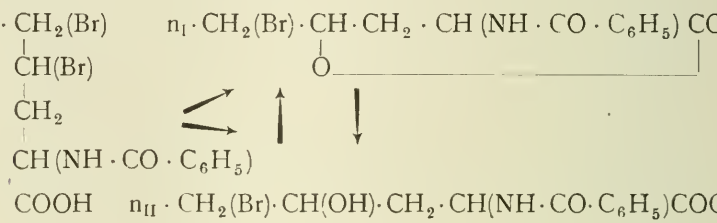
marie 40°)[1]), pour faire ensuite dissoudre le résidu dans 800 cc. d'alcool à 93 pour cent. L'addition d'hydroxyde de baryte en proportion correspondant au groupe carboxyle (625 cc. d'eau de baryte saturée à froid) détermina alors la précipitation d'une matière huileuse, qui toutefois par agitation rentra en dissolution. A la fin, le liquide présentait une légère opalescence et avait une réaction très nettement acide au tournesol. Abandonné jusqu'au lendemain, à la température du laboratoire, dans un ballon bien fermé, il déposa de gros cristaux lamellés (I), qu'on essora à la trompe et lava à 2 fois 25 cc. d'alcool à 80 pour cent. Après dessiccation à l'air, leur poids était de 11gr,7. L'évaporation dans le vide jusqu'au volume de 700 cc. environ de la liqueur filtrée et de l'alcool de lavage, engendra un dépôt notable qui, essoré à la trompe, présentait une couleur blanc jaunâtre et une consistance plutôt pâteuse. Lavé à la trompe avec 3 fois 25 cc. d'alcool à 60 pour cent, cette masse fut délayée dans une capsule avec 75 cc. d'alcool de même concentration, puis essorée de nouveau. Finalement, on la lava avec 3 fois 25 cc. d'alcool à 93 pour cent. Bien qu'à la suite de ce traitement cette préparation (II) fût devenue d'un blanc pur, elle n'était pas encore tout à fait pure, ainsi que le montre l'analyse; pour achever de la purifier, elle fut soumise à une nouvelle cristallisation par dissolution dans 150 cc. d'alcool à 80 pour cent bouillant, pour être le lendemain essorée et lavée avec 2 fois 25 cc. d'alcool. Séchée à l'air, la préparation finale (III) pesait 13gr,0. Le rendement total était de 24gr,7, soit environ 33 pour cent du calcul. — Tous les liquides de lavage et eaux-mères provenant de la préparation de la lactone, peuvent servir à la préparation de l'*α*-benzoylamino-*γ*-*δ*-dioxyvalérolactone (voir le paragraphe traitant de celle-ci).

La *δ*-bromelactone peut aussi s'obtenir en traitant l'«acide dibromé» impur (de consistance huileuse) par l'eau bouillante, qui le dissout petit à petit, la réaction devenant fortement acide par suite de la séparation de bromure d'hydrogène. La bromelactone cristallise directement de cette solution aqueuse. Pour la préparation de la lactone cette méthode n'est cependant guère pratique, parce qu'elle donne un rendement peu satisfaisant et

[1]) Il importe de chasser le chloroforme aussi complètement que possible, parce que autrement le traitement subséquent par l'hydroxyde de baryte en solution alcoolique donnera naissance, entre autres produits, à de l'acide formique et à du chlorure de baryum.

que, en outre, elle présente l'inconvénient de nécessiter l'emploi de très fortes quantités d'eau bouillante pour faire entrer en dissolution le composé dibromé. — On peut également préparer la bromelactone en faisant réagir sur le composé dibromé des sels d'argent ou des alcalis en proportion équivalente au groupe carboxyle.

En ce qui concerne la cause qui fait que le rendement en bromelactone reste inférieur à 30 pour cent de la proportion théorique, elle réside peut-être dans la formation simultanée d'un γ-oxyacide bromé en δ engendré, soit directement par le composé dibromé, soit indirectement par la bromelactone:



Je n'ai toutefois pas isolé cet oxyacide bromé[1]), de sorte que l'explication que je viens d'avancer reste une pure hypothèse.

L'analyse de l'α-benzoylamino-δ-brome-γ-oxyvalérolactone a conduit aux résultats suivants:

I. $0^{gr},2885$ a donné, d'après Carius, $0^{gr},1817$ AgBr. Dans le dosage de l'azote suivant Kjeldahl, $0^{gr},2335$ correspondait à $10^{cc},95$ d'hyposulfite N/14,01.

II. $0^{gr},1727$ a donné $0^{gr},1107$ AgBr; $0^{gr},1272$ correspondait à $5^{cc},7$ d'hyposulfit N/14.01.

III. $0^{gr},1401$ a donné $0^{gr},0879$ AgBr; $0^{gr},1993$ correspondait à $9^{cc},36$ d'hyposulfite N/14,01.

	Calculé	Trouvé
I.	Br 26,83 N 4,70	Br 26,81 N 4,68
II.	Br	26,60 N 4,48
III.	Br	26,70 N 4,70

On prépara la lactone IV à partir de l'acide «dibromallylhippurique», en ajoutant à la solution alcoolique de celui-ci un équivalent de sulfate d'argent dissous dans l'eau chaude. On fit subir à la préparation une nouvelle cristallisation dans le benzène.

IV. $0^{gr},1202$ ont donné $0^{gr},0759$ AgBr (26,90 % Br); $0^{gr},2526$ correspondaient à $11^{cc},8$ d'hyposulfite N/14.01 (4,67 % N).

La lactone V fut préparée en traitant l'acide dibromé par l'eau bouillante. Recristallisation de la préparation dans de l'eau.

V. $0^{gr},1154$ ont donné $0^{gr},0735$ AgBr (27,13 % Br); $0^{gr},1370$ correspondaient à $6^{cc},30$ d'hyposulfite N/14,01 (4,60 % N).

La bromelactone fond au «bloc Maquenne» (5 secondes) respectivement à 163° (I), à 162°,5 (IV) et à 162°,5 (V). Elle est peu soluble dans l'alcool à la température ordinaire du laboratoire; elle s'y dissout plus facilement, si l'on porte à l'ébullition. Dans le benzène et le chloroforme sa solubilité est à peu près la même. Elle n'est guère soluble dans l'éther, tandis que l'eau bouillante la dissout; mais en même temps elle subit une modification, en ce sens que la réaction devient acide et qu'il s'en sépare du bromure d'hydrogène. Le nitrate d'argent ne permet pas de déceler le brome au sein de l'alcool étendu à la température de la chambre; c'est seulement par ébullition en contact avec un sel d'argent qu'il se dépose petit à petit du bromure d'argent. La solution dans l'alcool dilué, préparée à la température du laboratoire, a une réaction neutre.

α-benzoylamino-γ-δ-dioxyvalérolactone.

$$CH_2(OH) \cdot CH \cdot CH_2 \cdot CH(NH \cdot CO \cdot C_6H_5) \cdot CO$$
$$O \text{ (liaison lactonique entre CH et CO)}$$

On soumit à la bromuration 0,3 molécule (73 gr) d'acide allylhippurique (voir p. 231) et prépara la δ-bromelactone suivant le procédé indiqué plus haut. ce qui fournit $12 + 14^{gr},5$ de bromelactone pure, soit 29,6 % du calcul. Le filtratum et l'eau de lavage provenant de ces cristaux = B; la lactone cristallisée = A.

A 22 gr. de la lactone obtenue on ajouta, dans un flacon d'une capacité de 2 litres, un litre d'eau de baryte saturée à froid (environ 3 fois autant que les 2 équivalents qu'avait donnés le calcul), puis on agita le flacon à la machine pendant trois heures, jusqu'à dissolution complète. Le jour suivant on sépara par filtration un dépôt formé probablement en majeure partie de carbonate de baryum. Le volume du liquide filtré était de 1200 cc.

On en employa 5 cc. pour effectuer une titration d'après Volhard au point de vue du brome ionisable. Il fallut $30^{cc},75$ d'une solution de nitrate d'argent décime, soit, en tout, $736^{cc},8$ de Br normal (indiqué par la théorie $738^{cc},2$). Une petite prise de la solution principale fut précipitée par un excès de nitrate argenté en solution nitrique, puis, après filtration, additionnée de lessive de soude jusqu'à réaction alcaline, et, après l'avoir fait bouillir une minute, on l'acidifia fortement à l'aide d'acide nitrique. La solution demeura presque limpide, sauf une opalescence à peine sensible.

Le baryum fut ensuite éliminé à l'aide d'acide sulfurique en quantité justement nécessaire, et le brome, en chauffant au bain-marie bouillant, par un excès de carbonate d'argent ajouté par portions. Après avoir filtré à la trompe, on élimina soigneusement l'excès d'argent au moyen de l'acide chlorhydrique. Le liquide filtré à nouveau était limpide et ne présentait qu'une faible coloration jaune. Après évaporation dans le vide jusqu'à un volume de 50 cc. environ, on le transvasa, à l'aide d'un peu d'eau, dans un gobelet, qu'on plaça dans la glacière. Le lendemain on y trouva séparés des cristaux blancs (I), qu'on lava avec 2 fois 10 cc. d'eau glacée; séchés à l'air, ils pesaient $8^{gr},5$. L'analyse donna pour résultat qu'ils étaient constitués par de l'*α*-benzoylamino-*γ*-*δ*-dioxyvalérolactone presque pure.

La liqueur filtrée et l'eau de lavage provenant de I (F_1) furent complétées au volume de 100 cc. exactement, dont 2 cc., étendus avec 10 cc. d'eau, servirent à la titration en présence de phénolphtaléine au moyen d'une solution N/5 de NaOH; il en fallut $1^{cc},95$ pour déterminer une coloration rouge juste perceptible. On porta alors à l'ébullition, qui fit disparaître cette couleur. Une nouvelle addition, goutte par goutte, d'alcali à la solution chaude provoqua, pour chaque goutte, la réapparition de la teinte rouge, qui cependant par agitation disparut de nouveau avec une certaine lenteur. Après addition de $0^{cc},9$, le liquide était encore incolore; mais lorsqu'on y eut ajouté encore $1^{cc},1$ de solution d'alcali, et qu'ensuite on l'eut porté à l'ebullition, il acquit une couleur rouge persistante, et après refroidissement on effectua alors une titration en retour au moyen d'acide chlorhydrique N/5. On constata que, en sus des $1^{cc},95$ de solution N/5 d'hydroxyde de soude ajoutés à froid, il en avait fallu $0^{cc},95$ pour provoquer une réaction légèrement alcaline. — On peut avec une certaine probabilité admettre qu'aux deux phases de la titration Na était entré dans un groupe carboxyle — à la température ordinaire, dans un groupe préexistant, à température élevée, dans un groupe formé à la suite de la titration même, par rupture d'une liaison lactonique —, et, d'autre part, il est probable que dans le premier de ces deux cas le groupement carboxyle entre dans l'acide *α*-benzoylamino-*γ*-*δ*-dioxyvalérique, tandis que dans le second cas il est engagé dans la lactone de cet acide. On obtient dès lors $273^{mgr},2$ de N sous la forme de l'acide, $33^{mgr},1$ N sous celle de sa lactone; en d'autres termes, d'après le titrage accompli, les 100 cc. comprendraient $4^{gr},9$ d'acide et $2^{gr},23$ de «lactone», ou bien, si nous comptons la totalité de l'azote comme de la «lactone», $6^{gr},8$ de celle-ci. Le dosage de l'azote total d'après Kjeldahl accuse une proportion de $8^{mgr},46$ d'azote dans 2 cc., en tout 423 mgr. ou 7 gr. de «lactone». Ainsi qu'on le verra par la suite, on peut des 96 cc. restants isoler 5 gr. de la «lactone» cherchée. Que celle-ci puisse être dosée quantitativement au moyen d'une titration alcalimétrique,

c'est ce qui se trouve confirmé par des essais portant sur de la lactone pure (voir l'analyse).

Dans le but de convertir l'acide en lactone, on chauffa F_I (les 96 cc. restants) pendant 5 heures, en présence d'un volume égal d'acide sulfurique normal aux $^2/_5$, dans un matras muni d'un réfrigérant à reflux et placé au bain-marie. Ensuite, après avoir éliminé tout l'acide sulfurique à l'aide d'eau de baryte, on rapprocha la liqueur filtrée dans le vide jusqu'à un volume de 20 cc. environ. Ce résidu fut transvasé, à l'aide d'eau, dans un gobelet, qu'on fit séjourner deux jours dans la glacière, où des agitations fréquentes accélérèrent la formation d'un certain nombre de cristaux que, essorés à la trompe, on lava avec 3 fois 5 cc. d'eau glacée. On obtint ainsi $4^{gr},7$ de cristaux (II) presque parfaitement blancs. Après évaporation dans le vide de la liqueur filtrée et de l'eau de lavage (F_{II}), on fit dissoudre le résidu huileux dans 60 cc. d'acide sulfurique normal aux $^2/_5$, puis on le chauffa durant dix heures au bain-marie bouillant (réfrigérant à reflux), pour agiter ensuite le liquide, qui présentait une coloration assez foncée, avec 2 fois 25 cc. d'éther. Ce dernier, soumis à l'évaporation, ne laissa qu'une faible quantité d'huile de couleur sombre.

Le dosage de l'azote faisant partie de la solution épuisée à l'éther, montra que l'azote total se montait à $182^{mgr},8$, celui titrable au formol à 100 mgr.

Par conséquent, il y aura eu séparation partielle du groupement benzoyle, et probablement aussi d'autres scissions. On élimina l'acide sulfurique au moyen d'une solution de baryte, puis évapora dans le vide la liqueur filtrée jusqu'à un volume très petit. Deux jours plus tard, on sépara par filtration quelques cristaux qui, après lavage avec 3 fois 2 cc. d'eau glacée suivi d'une dessiccation à l'air, pesaient $0^{gr},3$ (III). Le rendement total se chiffrait ainsi à $8,5 + 4,7 + 0,3 = 13^{gr},5$, soit 77,6 pour cent de la valeur calculée. I + II fut recristallisé dans de l'eau, ce qui donna le produit désigné dans la suite par La_I, et III recristallisé dnns 5 cc. d'eau fournit La_{II}.

Dans un second essai, on soumit $12^{gr},0$ de δ-bromelactone au traitement par 250 cc. de solution normale aux $^2/_5$ d'hydroxyde de baryum (2 équivalents = 200 cc.) suivant le même procédé qu'auparavant. La solution débarrassée de baryum et de brome (a) fut complétée jusqu'au volume de 300 cc.

On en employa 10 cc. pour la titration alcalimétrique en se

servant de la phénolphtaléine comme indicateur. A la température ordinaire il fallut $5^{cc},7$ de lessive de soude N/5, et après chauffage encore $1^{cc},3$, correspondant — d'après un calcul analogue à celui fait à l'occasion de l'essai précédent — à $8^{gr},6$, d'acide et $1^{gr},8$ de sa lactone ($9^{gr},8$ de lactone, tandis que la théorie n'avait indiqué que $9^{gr},4$).

Ce qui restait de (a) fut évaporé dans le vide jusqu'à obtention d'une huile tenace incolore, accusant une réaction fortement acide au tournesol. Après dissolution dans l'alcool, en proportion aussi petite que possible, on la versa dans un gobelet, qu'on plaça dans le vide au-dessus d'acide sulfurique concentré. Au bout de six jours — pendant lesquels on retira journellement le gobelet pour en remuer le contenu avec une baguette de verre —, l'huile s'était solidifiée de façon à former un gâteau cristallin de consistance légèrement pâteuse, et qui pourtant se laissait sans trop de peine réduire en poudre. Il fut dissous dans de l'eau jusqu'au volume de 100 cc. (b).

2 cc. de cette solution, étendus avec 10 cc. d'eau, demandèrent à la température ordinaire $1^{cc},9$ de lessive de soude N/5 pour produire une réaction légèrement alcaline en présence de la phénolphtaléine, et à température plus élevée, encore 2 cc. Rien que par le traitement dans le vide, la proportion de la lactone à l'acide s'est donc élevée de $^1/_5$ environ à 1; autrement dit, la proportion de lactone, qui formait primitivement 18,6 % de la quantité titrable à l'alcali, a monté à 51,2 %.

Le reste de (b), évaporé dans le vide vers un volume de 15 cc., donna $3^{gr},9$; la liqueur filtrée et l'eau de lavage, traitées par l'acide sulfurique normal au $^1/_5$, fournirent $4^{gr},8$; donc, en tout, $7^{gr},7$, ou 81 pour cent du rendement théorique.

Après avoir complété à 2 litres le filtratum B provenant de la δ-bromelactone A (voir p. 234), on en employa 1 litre (B_I) à la préparation de l'α-benzoylamino-γ-δ-dioxyvalérolactone; les 1000 cc. qui restaient (B_{II}) servirent à celle de la γ-oxyproline.

5 cc. de B_I contenaient $7^{mgr},3$ d'azote, de sorte que la teneur totale en azote se montait à 1464 mgr. La teneur en azote de (B) s'élevait donc à 2928 mgr., tandis que A se trouva en renfermer 1246 mgr., ce qui fait en tout 4174 mgr. La valeur théorique, calculée d'après la quantité, pesée d'une façon approximative, de l'acide allylhippurique mise en œuvre, serait de 4203 mgr. N.

A B_I on ajouta, à la température du laboratoire, 1 litre d'eau de baryte saturée à froid (calculé 2 equivalents = 524 cc.).

Le précipité produit en premier lieu (à la suite de l'addition d'eau) entra immédiatement en dissolution par agitation. Il se

dégagea une odeur assez caractéristique, rappelant celle de l'éther éthylbenzoïque, et en même temps on put en percevoir une plus âpre (provenant peut-être de bromure de benzoyle).

Après cinq jours d'abandon, dans un matras bien fermé, à la température de la chambre, un échantillon prélevé sur la solution limpide et soumis à la titration (d'après Volhard) relative au brome ionisable, permit de constater que la totalité de brome l'était bien; en d'autres termes, la réaction entre le baryum et le brome se montra complète.

Le baryum et le brome furent alors éliminés, celui-là par addition d'acide sulfurique, celui-ci au moyen de carbonate d'argent (sans filtration préalable); puis on filtra à la trompe, pour laver ensuite le précipité encore une fois avec 100 cc. d'eau ayant la température de la chambre.

Le précipité formé de bromure d'argent et de sulfate de baryte accusa, d'après Kjeldahl, une teneur en azote de 237 mgr., qui pouvait en majeure partie être extrait par lavage à l'eau chaude, opération dont on se dispensa pourtant parce que l'azote en question semble entrer dans la composition de substances fortement colorées et qui se déposent sous forme d'une huile dans la solution aqueuse dès que celle-ci se sera refroidie.

De la liqueur filtrée et de l'eau de lavage, on élimina au moyen d'acide chlorhydrique un excès d'argent. La solution limpide, qui accusait une réaction acide au tournesol et ne présentait qu'une faible teinte jaune, fut évaporée dans le vide jusque vers 700 cc., puis complétée à un litre.

On lui enleva 5 cc. en vue du dosage de l'azote d'après Kjeldahl, une proportion égale pour le titrage au formol, enfin également 5 cc. pour le titrage à l'alcali. L'azote total se chiffra par 1215 mgr., celui titré au formol par 519 mgr. La titration alcalimétrique demanda à la température ordinaire $1^{cc},06$ de NaOH N/5, à température plus élevée $0^{cc},45$ en plus.

De ces résultats il ressort que $4^{gr},5$ environ d'acide benzoïque (correspondant à 519 mgr. d'azote) se sont séparés du groupe aminé, et que seulement un peu plus de la moitié de la quantité totale de l'azote semble — d'après le titrage — faire partie du composé benzoylique du dioxyacide et de sa lactone (845 mgr. de N).

Les 985 cc. qui restaient (B) furent maintenant extraits par 3 fois 200 cc. d'éther, et chaque portion d'éther lavée avec 25 cc. d'eau. La solution épuisée à l'éther et l'eau de lavage combinées seront toujours désignées par B_1.

Les extraits éthérés réunis furent séchés au chlorure de calcium. On chassa l'éther et obtint pour résidu $6^{gr},73$ d'une matière cristal-

lisée, d'une coloration très foncée. On la fit dissoudre dans l'eau bouillante en quantité juste nécessaire, pour en séparer par filtration, le jour suivant, des cristaux d'un blanc parfait (I). La liqueur filtrée fut épuisée trois fois avec 50 cc. d'éther, et les extraits éthérés, soumis à l'évaporation, laissèrent pour résidu une matière cristalline, mais d'un blanc impur (II). I et II réunis et séchés à l'air avaient un poids de $5^{gr},1$, dont $0^{gr},5$ s'est montré contenir $5^{mgr},04$ d'azote, de sorte que la portion entière en contenait $51^{mgr},4$. En supposant que cet azote entre dans la composition d'une substance contenant environ 6 % d'azote (le dioxyacide 5,56 %, sa lactone 5,96 %), on arriverait à $0^{gr},44$ de substance azotée. En ce cas on trouverait $5^{gr},1 \div 0^{gr},44 = 4^{gr},66$ d'acide benzoïque, ce qui est un peu plus de la valeur calculée : $4^{gr},52$.

La solution épuisée à l'éther (B_I) fut alors évaporée dans le vide jusqu'à volume très faible (15 à 20 cc.), après quoi, renfermée dans un petit gobelet, elle subit la cristallisation dans la glacière. Au bout de deux jours, on essora les cristaux formés, puis les lava avec 3 fois 5 cc. d'eau glacée. Séchés à l'air, leur poids était de 5 gr. Cependant, n'étant pas parfaitement blancs, ils furent recristallisés dans 25 cc. d'eau, puis lavés avec 3 fois 5 cc. d'eau glacée. Après dessiccation à l'air, ils pesaient alors $4^{gr},2$ (La_{III}). — Aux eaux-mères on joignit les eaux de lavage provenant de cette préparation, pour les chauffer ensuite pendant cinq heures au bain-marie, avec un égal volume d'acide sulfurique normal aux $^2/_5$, de la manière précédemment décrite; on obtint ainsi des cristaux qui, lavés avec 3 fois 2 cc. d'eau glacée etc., pesaient $0^{gr},9$ (a). L'eau-mère jointe à celle de lavage fut soumise au même traitement encore une fois, ce qui fournit $0^{gr},5$ de cristaux (b). Recristallisés dans 10 cc. d'eau, a + b donna la préparation désignée dans l'analyse par La_{IV}. La liqueur filtrée et l'eau de lavage provenant de b réunies à celles de La_{IV}, furent complétées à 100 cc. («Reste B_I»).

2 cc. du «Reste B_I», titrés au formol, demandèrent $3^{cc},56$ de NaOH N/5, ce qui correspond à 498 mgr. de N dans la portion entière.

Le «Reste B_I» fut alors rapproché dans le vide jusque vers 5 cc., puis additionné, par portions, d'un grand excès d'alcool. Après deux jours, on en sépara par filtration quelques cristaux d'un blanc pur qui, lavés avec 3 fois 10 cc. d'alcool et séchés à l'air, pesaient $1^{gr},6$. On les reprit dans la quantité d'eau juste suffisante et les précipita à l'alcool. Les ayant reprécipités 3 fois de cette manière, on les sécha dans le vide sulfurique.

0gr,0921 correspondait à 9cc,78 d'hyposulfite N 14,01 (10,62 % N; oxyproline: 10,69 %). Le point de fusion au «bloc Maquenne» (5 sec.) était de 261—262°; celui de la γ-oxyproline est, d'après Leuchs, de 261° (corr.). Le goût était très doux.

La préparation était donc constituée par la γ-oxyproline. La manière dont ce corps prend naissance se trouve décrite dans l'introduction au présent mémoire et aussi plus loin (synthèse, p. 248—251).

Après évaporation à petit volume (5 cc. environ) de la lessive-mère provenant de l'oxyproline, on l'étendit avec de l'eau jusqu'à 50 cc., puis on la chauffa trois quarts d'heure au bain-marie bouillant, en contact avec un grand excès de carbonate de cuivre ajouté par portions. La solution ainsi obtenue et qui était d'un bleu foncé, fut filtrée à la trompe pour en séparer du carbonate cuivrique non dissous, puis on la rapprocha dans le vide. Le résidu, dont le volume ne dépassait guère 10 cc., fut mis avec une très faible proportion d'eau dans un petit gobelet. L'ayant abandonné dans la glacière pendant deux jours, on y trouva des cristaux bleus d'un sel cuivrique, qu'on essora à la trompe. Après les avoir lavés d'abord avec 15 fois 2 cc. d'eau glacée, puis avec 2 fois 10 cc. d'alcool à 93 pour cent, on les fit bouillir dans un gobelet avec 100 cc. d'alcool absolu. La préparation ainsi obtenue, qui après dessiccation dans l'air, pesait 2 gr., fut recristallisée dans 15 d'eau et lavée avec 3 fois 2 cc. d'eau.

Séché à l'air, 1gr,0250 de cette préparation perdit dans le vide à 75° 0gr,0205. 0gr,1059 donna 0gr,0238 CuO. Dans le dosage de l'azote d'après Kjeldahl, 0gr,2055 correspondait à 16cc,35 d'hyposulfite N/14,01.

	Calculé pour le sel cuivrique de l'oxpyroline	de l'oxyacide	Trouvé
N	8,66	7,79	8,12
Cu	19,61	17,68	18,35
H_2O (sel hydraté)	18,21	—	2,00

Ainsi qu'il ressort de l'analyse, la composition de cette préparation ne correspond ni au sel cuivrique de l'oxyproline ni à celui du dioxyacide. Une recristallisation réitérée dans de l'eau n'amena pas de modification appréciable dans la composition. Après tout, il paraît vraisemblable que la préparation est constituée par un mélange de plusieurs sels cuivriques ou bien par un sel cuivrique mélangé d'impuretés diverses.

L'analyse relative à La_I, La_{II}, La_{III}, et La_{IV} a donné les résultats que voici:

La_I: $0^{gr},1040$ donna $0^{gr},0523$ H_2O et $0^{gr},2333$ CO_2; $0^{gr},1571$, dosé d'après Kjeldahl, correspondait a $9^{cc},41$, et $0^{gr},1277$ dosé également, à $7^{cc},70$ d'hyposulfite N/14,01; $1^{gr},4840$ ont perdu dans le vide à 75^0 $0^{mgr},4$.

		Calculé	Trouvé	
C_{12}	144	61,24	61,18	
H_{13}	13,1	5,57	5,62	
O_4	64	27,23	—	
N	14,01	5,96	5,99	6,03
	235,11	100,00		

Une solution aqueuse de $0^{gr},0987$ dans 10 cc. accusa une réaction neutre, et l'addition de phénolphtaléine et d'une goutte de solution norm. au $^1/_5$ de NaOH lui fit prendre instantanément une coloration rouge. Celle-ci cependant disparut par chauffage. On ajouta alors 5 cc. de solution norm. au $^1/_5$ de NaOH, puis porta de nouveau le liquide à l'ébullition. Après refroidissement, on titra en retour au moyen d'acide chlorhydrique. On constata que l'ébullition avait fait disparaître $2^{cc},1$ de NaOH N/5, et que, d'après la théorie, la rupture de la liaison lactonique et la formation du sel sodique du dioxyacide en exigent $2^{cc},1$.

La_{II}: $0^{gr},1531$ de cette préparation correspondait, dans le dosage de l'azote d'après Kjeldahl à $9^{cc},29$ d'hyposulfite N/14,01. La titration avec chauffage de $0^{gr},1136$ demanda $2^{cc},45$ de NaOH N/5.

La_{III}: Dans le dosage d'azote suivant Kjeldahl, $0^{gr},1245$ correspondait à $7^{cc},47$ d'hyposulfite N/1401; $0^{gr},1078$ de même, à $6^{cc},49$. La titration à chaud de $0^{gr},1333$ demanda $2^{cc},83$ de NaOH N/5.

La_{IV}: $0^{gr},0836$, dosé d'après Kjeldahl, correspondait à $5^{cc},10$ d'hyposulfite N/14,01, et $0^{gr},0735$ demanda par la titration à chaud $1^{cc},51$ de NaOH N/5.

		NaOH N/5 cc.	N %	
Calculé:	La_{II}	2,42		
	La_{III}	2,83	5,96	
	La_{IV}	1,56		
Trouvé:	La_{II}	2,45	6,07	
	La_{III}	2,83	6,00	— 5,97
	La_{IV}	1,51	6,10	

α-benzoylamino-*γ*-*δ*-dioxyvalérolactone fond («bloc Maquenne» 5 secondes) entre 167^0 et 168^0. — Quoique facilement soluble dans l'alcool, elle peut y être recristallisée, bien qu'avec une perte assez considérable. Il vaut mieux la recristalliser dans l'eau, parce que dans ce milieu elle est difficilement soluble à froid et très facilement à chaud; l'éther ne la dissout que très difficilement. La solution aqueuse de cette lactone a une ré-

action neutre et ne contient pas d'azote titrable au formol. La liaison lactonique peut être titrée quantitativement à l'aide d'un alcali à la température d'ébullition (solution aqueuse). Cristallise dans la solution aqueuse en forme d'aiguilles ou tablettes minces rectangulaires.

A partir de la δ-brome-γ-oxylactone benzoylée, on obtint un rendement en dioxylactone benzoylée de 81 pour cent de la valeur théorique. Calculé sur l'acide allylhippurique — c'est-à-dire en réunissant les préparations tirées de la δ-brome-γ-oxylactone benzoylée à celles tirées de B_I —, le rendement ne se montait qu'à 29 pour cent de la théorie. A + B_I contenait 2710 mgr. d'azote; A a fourni $7^{gr},7$, B $5^{gr},6$ de la dioxylactone benzoylée; en tout, $13^{gr},3$ contenant 798 mgr. d'azote.

Sel chlorhydrique de l'α-amino-γ-δ-dioxyvalérolactone.

$$(CH_2(OH) \cdot CH \cdot CH_2 \cdot CH(NH_2) \cdot CO)\,HCl$$
$$\qquad\qquad O$$

On chauffa pendant cinq heures au bain-marie bouillant $8^{gr},5$ de la dioxylactone benzoylée avec 100 cc. d'acide chlorhydrique fort (38 %), dans lequel la dite combination se dissout facilement à chaud. Le liquide prit une coloration faiblement jaunâtre. Il y eut sublimation d'acide benzoïque, qui se condensa sous forme de cristaux dans la partie froide du col du matras. Après refroidissement, la solution limpide, avec quelques cristaux d'acide benzoïque, fut étendue avec un égal volume d'eau. puis épuisée avec 3 fois 100 cc. d'éther, et chacune de ces portions d'éther fut lavée avec 2 fois 10 cc. d'eau. Le mélange de A et de cette eau de lavage fut ensuite évaporé dans le vide jusqu'à consistance huileuse. Après dissolution du résidu dans 200 cc. d'eau, on rapprocha de nouveau dans le vide, puis, à l'aide de petites doses d'alcool, on transvasa le nouveau résidu, également huileux, dans un gobelet, où l'on y ajouta un fort excès d'alcool. Au bout de quelques heures, le liquide fit tomber la dioxylactone chlorhydrique dépourvue de benzoyle, en forme de cristaux blancs. Le lendemain, on essora à la trompe et lava avec une abondante quantité d'alcool (Liqueur filtrée + alcool de lavage = F_I). Les cristaux parfaitement blancs pesaient $3^{gr},9$ (I). On les fit dissoudre dans 7 cc. d'eau, pour les reprécipiter ensuite par addition successive de fortes doses d'alcool. Les cristaux II furent lavés à l'alcool. (Filtratum + alcool de lavage = F_{II}).

$F_I + F_{II}$ fut rapproché dans le vide jusque vers 30 cc., que l'on compléta avec de l'eau à 200 cc. On élimina l'acide chlorhydrique en agitant, à la température du laboratoire, en présence de carbonate d'argent, et un excès d'argent fut enlevé par addition d'acide chlorhydrique. La solution accusa une réaction acide au tournesol, due probablement au dioxyacide benzoylé, auquel aurait donné naissance la lactone correspondante sous l'influence du carbonate d'argent.

Il s'ensuit donc que l'élimination du groupe benzoyle n'était pas complète après cinq heures de chauffe avec l'acide chlorhydrique. Néanmoins, je crus devoir préférer une action de courte durée, résultant en un enlèvement incomplet du groupe benzoyle, plutôt que de courir le risque, comporté par une action prolongée, d'arriver à une formation peut-être considérable de γ-oxyproline.

La solution débarrassée d'acide chlorhydrique et d'argent fut rapprochée dans le vide à 100 cc., puis additionnée d'une égale quantité d'acide sulfurique normal aux $^2/_5$, après quoi on la chauffa à reflux pendant cinq heures au bain d'eau bouillante, ce qui eut pour effet de la foncer considérablement. Après agitation de la solution en présence d'un peu de noir animal, puis élimination de l'acide sulfurique au moyen d'eau de baryte, on évapora la solution filtrée vers 10 cc., pour l'abandonner ensuite à la cristallisation dans un gobelet. Au bout de deux jours, on sépara à la trompe quelques cristaux de couleur assez foncée qui, lavés à 2 fois 3 cc. d'eau glacée, puis séchés à l'air, pesaient $0^{gr},7$. Le filtratum et l'eau de lavage de ces cristaux furent laissés de côté. Recristallisés dans une faible quantité d'eau, ils présentaient une couleur d'un blanc pur.

Dans la préparation ainsi obtenue, le dosage de l'azote d'après Kjeldahl donna pour résultat que $0^{gr},1356$ correspondaient à $8^{cc},27$ d'hyposulfite N/14,01 (6,1 % N). La solution aqueuse de la préparation accusait une réaction neutre et put être titrée comme une solution de lactone. Elle ne contenait pas d'azote titrable au formol.

$8^{gr},5$ de benzoyldioxylactone mise en œuvre $\div$ $0^{gr},7$ récupéré $= 7^{gr},8$ de matière première, qui doivent donner $4^{gr},9$ de dioxylactone chlorhydrique. On obtint $3^{gr},9$, soit environ 80 pour cent du rendement calculé.

Analyse portant sur la dioxylactone chlorhydrique reprécipitée:

$0^{gr},1093$ correspondait, dans le dosage d'azote d'après Kjeldahl, à $9^{cc},14$ d'hyposulfite N/14,01; dans la titration suivant Volhard, $0^{gr},1364$ exigea $8^{cc},17$ de AgNO décime.

Calculé.....	N : 8,36 %	HCl : 21,29 %
Trouvé.....	8,36 %	21,83 %

La α-amino-γ-δ-dioxyvalérolactone chlorhydrique fond, en se décomposant, à 189—193° («Bloc Maquenne», 5 secondes). Elle est très facilement soluble dans l'eau, tandis que dans l'alcool et dans l'éther elle ne se dissout guère. Précipitée d'une solution aqueuse au moyen de l'alcool, elle s'obtient à l'état d'aiguilles bien développées et agrégées en forme de bobines.

Sel cuivrique de l'acide α-amino-γ-δ-dioxyvalérique.

$(CH_2\,(OH)\cdot CH(OH)\cdot CH_2\cdot CH(NH_2)\cdot COO)_2\,Cu.$

2 gr. de la dioxylactone chlorhydrique reprécipitée furent débarrassés de chlore par agitation de la solution aqueuse à la température ambiante en présence d'un excès de carbonate d'argent. Le liquide prit bientôt une réaction fortement alcaline et fut filtré à la trompe. Le précipité, conjointement avec l'excès de carbonate d'argent, fut lavé soigneusement à l'eau. La liqueur filtrée renfermait d'abondantes quantités d'argent, qu'on élimina au moyen d'acide chlorhydrique. Après précipitation de la totalité de l'argent, la solution filtrée n'avait qu'une réaction faiblement alcaline au tournesol, probablement parce que la majeure partie de la liaison lactonique avait été rompue par le carbonate d'argent. On chauffa alors la liqueur claire incolore pendant trois quarts d'heure au bain d'eau bouillante, en ajoutant par portions un grand excès de carbonate de cuivre. Par filtration à la trompe, la liqueur, qui avait pris une couleur bleu foncé, fut dépourvue de carbonate cuivrique indissous, puis évaporée dans le vide vers 30 cc. Après abandon de ce résidu dans un gobelet placé jusqu'au lendemain dans la glacière, il s'était formé quelques cristaux bleu clair qui, séparés par filtration, furent lavés à 2 fois 2 cc. d'eau glacée (Vu leur faible quantité, on ne les examina cependant pas de plus près). La liqueur filtrée, évaporée au bain-marie vers 20 cc., fut additionnée, par portions, d'un excès d'alcool, qui détermina immédiatement la précipitation d'une matière bleu clair qui, le lendemain, fut séparée par filtration et lavée à 3 fois 2 cc. d'eau, 3 fois 5 cc. d'alcool à 50 pour cent, enfin 3 fois 10 cc. d'alcool à 97 pour cent. Séchée à l'air, la préparation offrait une couleur d'un azur clair et pesait $1^{gr},4$, soit environ 65 pour cent du calcul. L'eau-mère provenant de

ce sel cuivrique, et que je n'ai pas jusqu'ici soumise à un examen plus approfondi, présentait une teinte bleu foncé.

On fit dissoudre le sel cuivrique dans 20 cc d'eau bouillante, pour le précipiter de nouveau au moyen de l'alcool. C'est ce précipité qui, séché à l'air, fut soumis à l'analyse.

$0^{gr},4638$ perdit dans le vide à 80^0 $6^{mgr},3$. Dans le dosage d'azote suivant Kjeldahl $0^{gr},0921$ correspondait à $7^{cc},08$, et $0^{gr},1176$ de même, à $9^{cc},00$ d'hyposulfite N/14,01; $0^{gr},2113$ ont donné $0^{gr},0238$ CuO.

	Calculé	Trouvé	
N	7,79	7,79	7,75
Cu.....	17,64	17.53	

Une autre préparation du sel cuivrique du dioxyacide fut préparée sans isolation et purification préalables de la dioxylactone chlorhydrique. Après traitement de 10 gr. de la dioxylactone benzoylée par l'acide chlorhydrique concentré durant dix heures au bain d'eau bouillante, l'acide benzoïque fut extrait à l'éther, puis on évapora la liqueur acide deux fois dans le vide, pour faire dissoudre ensuite le résidu dans de l'eau et éliminer l'acide chlorhydrique au moyen du carbonate d'argent. On traita par le carbonate de cuivre la liqueur filtrée dépourvue d'argent et d'acide chlorhydrique, puis on rapprocha vers 50 cc. la solution du sel cuivrique, ce qui donna lieu à une faible cristallisation (du sel cuivrique de l'oxyproline?). On soumit le filtratum à une forte évaporation au bain-marie, puis on précipita le résidu au moyen de l'alcool. Le sel cuivrique bleu clair ainsi obtenu fut reprécipité de l'eau par l'alcool une fois (I), deux fois (II), trois fois (III).

I. $0^{gr},6314$ perdit dans le vide à 75^0 $0^{gr},0201$; $0^{gr},1389$ correspondait, dans le dosage d'azote d'après Kjeldahl, à $10^{cc},76$ d'hyposulfite N/14,01.

II. $0^{gr},7450$ perdit dans le vide à 110^0 $0^{gr},0266$; $0^{gr},1703$ correspondait à $13^{cc},19$ d'hyposulfite N/14,01.

III. $0^{gr},2499$ perdit dans le vide à 75^0 $0^{gr},0067$; $0^{gr},1200$ correspondait à $9^{cc},28$ d'hyposulfite N/14,01; $0^{gr},1200$ donna $0^{gr},0263$ CuO.

	Calculé (sel anhydre)	Trouvé		
		Séché à l'air		Séché dans le vide
I.	7,79 % N	3,29 % H_2O	7,75 % N	7,99 % N
II.	—	3,57 —	7,74 —	8,02 —
III.	—	2,68 —	7,73 —	7,95 —
	17,64 % Cu	17,51 % Cu		17,99 % Cu

La diminution qu'a subie le poids par dessiccation dans le vide et qui se chiffre par 3 pour cent environ, est peut-être due à la présence d'eau de cristallisation dans une impureté constituée par un sel cuivrique de l'oxyproline. Il se peut, en effet, que ce dernier corps se soit formé à la suite du chauffage en présence d'acide chlorhydrique (chauffage qui durait dix heures), et qu'une faible quantité de ce sel cuivrique n'ait pas pu être éliminée par la recristallisation. D'ailleurs, la dessiccation fit prendre aux préparations une coloration vert gris assez foncé, indice d'une décomposition. Presque tout le poids perdu se récupéra pourtant à l'air humide. Lors de la préparation du sel cuivrique du dioxyacide à partir de la lactone chlorhydrique pure, on évita cependant ces impuretés qui sont si difficiles à interpréter.

Le sel cuivrique du dioxyacide est facilement soluble dans l'eau et peut y être recristallisé, bien qu'avec une perte considérable. Il ne se dissout point dans l'alcool et l'éther. (Les conditions de solubilité s'accordent donc avec celles du sel analogue préparé par Fischer et Krämer (l. c.).) La couleur de notre sel est d'un azur clair. L'alcool le précipite d'une solution aqueuse sous forme d'agglomérations arrondies d'aiguilles extrêmement fines. Fischer et Krämer décrivent leur préparation comme «... kleine Blättchen, die unter dem Mikroskop als ziemlich regelmässige sechseckige Formen erscheinen». Ces savants en donnent l'analyse suivante:

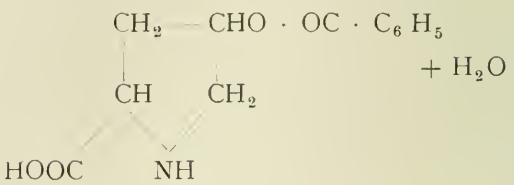
	Calculé	Trouvé			
N.....	7,79	7,84			
C.....	33,35	33,59			
H.....	5,60	5,36			
Cu.....	17,68	17,67	17,57	17,88	17,60

A ce propos ils font observer ceci: «Man sieht aus den Abweichungen im Kohlenstoff und im Wasserstoff, dass das Salz noch nicht ganz rein war. Aber die Differenzen sind nicht gross genug um die Formel ganz in Zweifel ziehen zu lassen». Ils ont préparé l'acide aminé même à partir du sel cuivrique, et ils ont établi que la teneur en carbone et en hydrogène de cet acide s'écartait également de la théorie.

Je rendrai plus loin compte des résultats préliminaires qu'ont donnés des essais commencés en vue d'isoler le dioxyacide et

d'élucider la manière dont il se comporte dans la formation de la lactone.

γ-oxybenzoylproline.



On chauffa pendant huit heures au bain d'eau bouillante dans un matras en verre d'Iéna, de la capacité de 3 litres et muni d'un réfrigérant à reflux, 1000 cc. (B_{II}), soit la moitié, du filtratum de l'α-benzoylamino-δ-brome-γ-oxylactone (p. 238), qu'on additionna chaque demi-heure de 5 gr. de carbonate de baryum, jusqu'à concurrence de 60 gr.; pendant les deux dernières heures on n'en ajouta donc plus. L'alcool ne tarda pas à entrer en ébullition, et la liqueur, de jaune paille clair qu'elle était au début, devint peu à peu d'une couleur nettement brune, en même temps que, déjà après l'addition de 4 fois 5 gr. dudit carbonate, la réaction, d'abord fortement acide, se changea en légèrement alcaline au tournesol. Vers la fin du chauffage, le contenu du matras montrait une tendance à faire des soubresauts.

Par filtration à la trompe à chaud, la liqueur fut débarrassée de carbonate barytique resté inaltéré, qu'on lava quelques fois avec l'alcool à 93 pour cent.

Une titration effectuée d'après Volhard, sur une prise de la liqueur filtrée, relativement au brome ionisable, donna pour résultat que 86 % de la quantité de brome se trouvaient dans cet état.

Après élimination du baryum au moyen de l'acide sulfurique, on chauffa (sans filtration préalable) au bain-marie, en ajoutant à plusieurs reprises du carbonate d'argent jusqu'à réaction faiblement alcaline, pour séparer ensuite par filtration à la trompe le précipité de bromure d'argent et de sulfate de baryum, qu'on lava à 3 fois 100 cc. d'eau.

D'après un dosage effectué sur une petite partie du précipité, la totalité de celui-ci contenait 233 mgr. d'azote. En ce qui concerne la perte ainsi constatée, il en est de même que pour celle observée précédemment dans des précipités semblables engendrés lors de la préparation de la dioxylactone benzoylée.

La liqueur filtrée et l'eau de lavage réunies furent évaporées jusque vers 100 cc. Il se déposa une huile (a) de couleur très

foncée, dont le liquide fut séparé par décantation, pour être complété avec de l'eau jusqu'au volume de 500 cc. (b). Je n'ai pas pu amener l'huile (a) à cristalliser. Elle renferme du brome non-ionisable, accuse une réaction acide au tournesol, se dissout difficilement dans l'eau, et cette solution la fait tomber apparemment inaltérée. L'alcool la dissout avec facilité, l'éther difficilement.

Un dosage d'azote portant sur 5 cc. de (b) permit de conclure à une teneur totale en azote de 925 mgr. seulement. B_{II} en contenait primitivement 1464 mgr. La perte d'azote s'élève à 539 mgr., dont 233 mgr. dans le précipité de bromure d'argent et de sulfate de baryum, 306 mgr. dans l'huile foncée (a). Sur les 925 mgr. d'azote, 526 mgr. sont — selon une détermination faite sur un petit échantillon — titrables au formol suivant Sørensen. Il ne peut guère être ici question d'une séparation hydrolytique d'acide benzoïque libre provoquée par la réaction faiblement alcaline régnant pendant le chauffage. La totalité de la liqueur fut cependant extraite à l'éther (3 fois 100 cc. d'éther, chaque portion étant lavée avec 25 cc. d'eau). Les extraits éthérés présentaient une couleur foncée, et ne laissèrent par évaporation qu'une huile également foncée. Celle-ci bouillie avec de l'eau s'y dissout en partie; mais la solution chaude ne fait tomber, par refroidissement, que de l'huile foncée et point de cristaux.

On peut en conclure que, si le groupe benzoyle s'est détaché à l'état d'acide benzoïque libre, cela ne s'est fait que dans une très faible étendue, et les 526 mgr. d'azote titrables au formol ne peuvent pas avoir pris naissance de cette manière. Il n'y a donc pas d'autre alternative possible que d'admettre une migration du groupe benzoyle dans la molécule, avec mise en liberté du groupe aminé. Vu la réaction neutre de la liqueur, il faut, en outre, admettre la présence d'un groupe carboxyle libre, supposition qui se trouve corroborée par ce fait que la solution renfermait une substance donnant lieu à une combinaison cuivrique.

On chauffa les 500 cc. de (b) pendant trois quarts d'heure dans une capsule de porcelaine placée au bain d'eau bouillante, en ajoutant par portions un fort excès de carbonate de cuivre. On vit tomber aussitôt des pelotes colorées en bleu très clair et formées très vraisemblablement de la *γ*-oxybenzoylproline cuivrique: $(C_5H_7O_3N \cdot OCC_6H_5)_2Cu$. Après refroidissement de la liqueur, on essora à la trompe le sel cuivrique difficilement soluble. qu'on lava ensuite avec une forte quantité d'eau. (Pour le traitement auquel on soumit la liqueur filtrée et l'eau de lavage (CuF), voir p. 255). La combinaison cuivrique est si difficilement soluble dans l'eau, qu'elle s'y laisse à peine recristalliser. Précipitée directement, elle ne se prête guère à l'analyse parce qu'elle

est mêlée d'une certaine proportion de carbonate de cuivre et que, conséquemment, elle donne à l'analyse une valeur trop élevée pour la teneur en cuivre et un chiffre trop peu élevé pour celle en azote.

$0^{gr},6350$ perdit dans le vide à 90^0 $3^{mgr},5$; $0^{gr},1153$ correspondait dans le dosage d'azote suivant Kjeldahl à $5^{cc},5$ d'hyposulfite N/14,01; $0^{gr},1051$ donna $0^{gr},0171$ Cu.

	Calculé	Trouvé
N.....	5,29	4,83
Cu.....	11,98	13,00

Le sel cuivrique séché à l'air pesait $11^{gr},5$ et renfermait, par conséquent, 555 mgr. d'azote, correspondant à $9^{gr},3$ d'oxyproline. Il fut mis en suspension dans 10 cc. d'acide sulfurique normal aux $^2/_5$ et chauffé dix minutes au bain d'eau bouillante, puis essoré à la trompe, ce qui fournit un liquide coloré en bleu et un résidu de sel cuivrique resté inattaqué. On traita ce résidu, de la même manière que la portion première, par 100 cc. d'acide sulfurique normal aux $^2/_5$, puis on l'essora. Soumis cinq fois à ce traitement, le sel cuivrique ne laissa sur le filtre qu'un reste extrêmement faible d'une couleur grisâtre. Les filtrats réunis furent débarrassés de cuivre à l'aide de sulfure d'hydrogène, et après séparation du sulfure cuivrique par filtration à la trompe, puis lavage avec d'abondantes quantités d'eau, on enleva le sulfure d'hydrogène au liquide en y faisant arriver un courant rapide d'air atmosphérique. Débarrassée ensuite d'acide sulfurique au moyen d'eau de baryte, la liqueur filtrée était limpide, incolore, et accusait une réaction neutre. La rapprochant alors dans le vide vers 50 cc., on vit tomber dans le liquide encore chaud des cristaux, qu'on fit rentrer en dissolution en chauffant légèrement au bain-marie, pour transvaser ensuite la solution dans un gobelet, qu'on installa dans la glacière. Le jour suivant, on essora à la trompe et lava à 3 fois 5 cc. d'eau glacée. Le poids des cristaux séchés à l'air (I) était de $5^{gr},1$. En évaporant l'eau-mère et l'eau de lavage vers 10 cc., on obtint des cristaux (II), qu'on lava à 3 fois 2 cc. d'eau et qui, séchés à l'air, pesaient $3^{gr},3$. L'eau mère et celle de lavage provenant de (II) furent évaporées dans le vide sulfurique jusqu'à consistance d'une bouillie, puis essorées à la trompe et lavées à 3 fois 1 cc. d'eau glacée, ce qui fit obtenir des cristaux (III) pesant $0^{gr},3$ après dessiccation à l'air. Le filtrat de (III) fournit par chauffage en présence de

carbonate de cuivre $0^{gr},45$ de sel cuivrique. I, II et III étaient d'un blanc presque pur tirant très légèrement sur le vert. Le rendement total s'élevait ainsi à $(5,1 + 3,3 + 0,3 =) 8^{gr},7$, c'est-à-dire environ 90 pour cent de la valeur calculée à partir du sel cuivrique. Calculé sur la proportion d'azote de la matière première (B_{II}): 33 pour cent de la théorie.

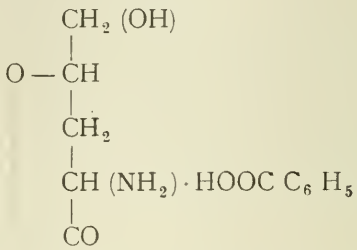
I. $5^{gr},23$ de la préparation obtenue furent chauffés au bain d'eau bouillante, pendant une heure, dans 100 cc. d'eau de baryte saturée à froid[1]). L'expérience que je viens de relater m'a permis de constater que la proportion calculée d'acide benzoïque est mise en liberté par ce traitement et peut être extraite à l'éther après élimination de la totalité du baryum au moyen de l'acide sulfurique. Effectivement, l'évaporation des extraits ainsi obtenus laissa $2^{gr},6$ de matière cristalline contenant, en total, $0^{mgr},5$ d'azote, le calcul ayant indiqué $2^{gr},52$ d'acide benzoïque. La solution extraite à l'éther (P) fut utilisée directement à la préparation des deux sels cuivriques de la γ-oxyproline (voir p. 253).

II. Dans une seconde expérience, je fis dissoudre, à la température ordinaire, $0^{gr},5421$ de la combinaison benzoylique dans 10 cc. d'eau + 10 cc. d'acide sulfurique normal aux $^2/_5$, puis j'opérai l'épuisement à 3 fois 50 cc. d'éther. Soumis à l'évaporation, les extraits étherés ne donnèrent pas de résidu.

Il résulte de ces deux expériences qu'un groupement benzoyle se trouve toujours dans la molécule (I) et en véritable liaison (II). Un titrage au formol montra qu'il y a un groupe amine ou imine libre et que, par conséquent, le groupement benzoyle ne peut y être lié. Ce résultat se trouve confirmé également par le traitement à l'eau de baryte, lequel a permis d'établir que le groupe benzoyle est lié d'une façon moins fixe qu'il ne l'est ordinairement dans les dérivés α-aminobenzoylés. Quant à l'hypothèse d'après laquelle un groupe benzoyle serait lié à la façon d'un anhydride à un groupement carboxyle, il n'en peut être question, vu le seul fait qu'une pareille combinaison devrait nécessairement avoir une réaction alcaline. Enfin, on ne saurait

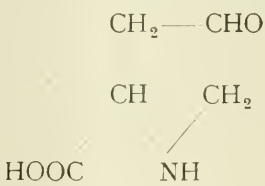
[1]) Il était à prévoir qu'un pareil traitement n'aurait pas pour effet de détacher, du moins pas d'une façon tant soit peu complète, un groupe benzoyle lié à un groupe aminé en position α; l'exactitude de cette prévision a été confirmée par mes propres essais portant sur la dioxylactone benzoylée, essais où il ne fut possible, par une heure de chauffe avec l'eau barytique, de détacher qu'une très minime proportion d'acide benzoïque. Comp. Sørensen: Ces mêmes Comptes-rendus, **10**, 121 (1911).

non plus expliquer toutes les propriétés de la substance en question en admettant une espèce de combinaison additive entre l'acide benzoïque et une dioxylactone:



Une pareille combinaison aurait sans doute une réaction neutre; mais l'acide benzoïque devrait évidemment pouvoir être extrait à l'éther après acidulation par l'acide sulfurique, ce qui n'est point le cas.

Ainsi donc, il ne reste pas d'autre explication plausible que celle qui consiste à admettre que le groupe benzoyle se trouve fixé, à la manière d'un éther-sel, au groupe oxy en position γ: $= CH \cdot O \cdot OCC_6H_5$. Il faudrait indubitablement assigner à la substance la formule de constitution suivante:



constitution qui s'accorde bien avec l'analyse. En partant de la γ-oxybenzoylproline, nous avons pu préparer les deux sels cuivriques de l'oxyproline ainsi que cette dernière elle-même (voir plus bas).

L'analyse de la γ-oxybenzoylproline préparée par une seule recristallisation, dans l'eau, de I, II et III réunis, a donné les résultats qui suivent:

A. Préparation séchée à l'air:

$0^{gr},7970$ perdit dans le vide à 70^0 $0^{gr},0522$; $0^{gr},1324$ correspondait, dosé d'après Kjeldahl, à $7^{cc},36$ d'hyposulfite N/14,01 et $0^{gr},1593$ de même à $8^{cc},89$.

B. Préparation desséchée dans le vide à 70^0:

$0^{gr},1814$ donna $0^{gr},0920$ H_2O et $0^{gr},4069$ CO_2; $0^{gr},1295$ correspondait à $7^{cc},3$, et $0^{gr},1571$ à $9^{cc},40$ d'hyposulfite N/14,01.

		B		A	
		Calculé	Trouvé	Calculé	Trouvé
C_{12}	144	61,25	61,17		
H_{13}	13,1	5,57	5,67		
O_4	64	27.22	—		
N	14,01	5,96	5,86 5,99	5,54	5,56 5,58
H_2O	—	—	—	7,12[1])	7,00[1])
	235,11	100,00			

0gr,1213 de matière séchée à l'air exigea dans la titration au formol 2cc,2 de solution normale au 1/5 de NaOH, correspondant à 5,08 % d'azote titrable au formol. Le calcul avait indiqué 5,56. Si le chiffre trouvé est sensiblement moins élevé, c'est sans doute simplement parce que, de même que la proline et l'oxyproline[2]), le dérivé considéré ne se prête pas à une titration suivant la méthode employée.

La γ-oxybenzoylproline fond au «bloc Maquenne» (5 secondes) à 158—161°. Elle se dissout facilement dans l'eau chaude, moins facilement dans l'eau froide (moyen de recristallisation) et dans l'alcool chaud; le chloroforme et le benzène bouillants la dissolvent difficilement, l'éther pas du tout. Elle est d'un goût très amer. Elle cristallise de l'alcool sous forme de longues aiguilles rectangulaires.

La γ-oxyproline et son sel cuivrique.

La solution neutre épuisée à l'éther (solution P, p. 251) fut chauffée pendant trois quarts d'heure au bain d'eau bouillante, en ajoutant par portions du carbonate cuivrique en excès, puis, après avoir filtré à la trompe la liqueur chaude bleu foncé, on évapora au bain-marie vers 30 cc., pour abandonner ensuite à la cristallisation dans la glacière. Le lendemain, on essora les cristaux séparés à la trompe, puis les lava à 5 fois 3 cc. d'eau glacée. Le sel cuivrique I a séché à l'air avait un poids de 5gr,1 et une couleur d'un bleu violet clair. L'eau-mère et l'eau de lavage provenant de ce sel furent rapprochées au bain-marie vers 10 cc. et additionnées d'alcool en fort excès. Au bout de quelques jours, on sépara par filtration un sel bleu violet foncé qui, lavé à l'alcool et desséché à l'air, pesait 0gr,7 II a. I a fut recristallisé dans de l'eau.

[1]) Calculé sur matière hydratée.

[2]) Ces mêmes Comptes-rendus, **7**, 37 (1907).

I a. 1 gr,3497 perdit dans le vide à 110° 0 gr,2416; 0 gr,1587 donna 0 gr,0388 CuO; 0 gr,3157 correspondit à 26 cc,75 d'hyposulfite N/14,01.

Calculé:	H_2O[1]) 18,21;	N 8,66;	Cu 19,61
Trouvé:	17,90;	8,53;	19,53

A partir du sel cuivrique, on prépara l'acide aminé libre en précipitant le cuivre à l'aide de l'hydrogène sulfuré, puis évaporant la liqueur filtrée et précipitant au moyen de l'alcool. Les cristaux obtenus furent repris dans un peu d'eau et reprécipités par l'alcool. Enfin la préparation fut séchée dans le vide sulfurique.

0 gr,1486 donna 0 gr,0923 H_2O et 0 gr,2500 CO_2; 0 gr,1460 correspondait à 15 cc,48 N/14,01 d'hyposulfite.

Calculé:	C 45,77;	H 6,92;	N 10,69
Trouvé:	45,88;	6,94;	10,60

L'oxyproline entra en fusion sur le «bloc Maquenne» (5 secondes) à 261—262° (d'après Leuchs 261°, corr.). Elle était d'un goût très doux.

II a. 0 gr,6226 perdit dans le vide à 110° 0 gr,0019; 0 gr,1148 correspondait à 9 cc,72 d'hyposulfite N/14,01; 0 gr,1581 donna 0 gr,0387 CuO.

Calculé:	N 8,66;	Cu 19,61
Trouvé:	8,47;	19,54

Dans une seconde expérience je préparai les sels cuivriques de l'oxyproline d'une manière analogue.

I b. 0 gr,6494 perdit dans le vide à 110° 0 gr,0491; 0 gr,3137 (matière anhydre) correspondait à 26 cc,75 d'hyposulfite N/14.01; 0 gr,1577 (substance anhydre) donna 0 gr,0388 CuO.

Calculé:	N 8,66;	Cu 19,61;	H_2O —
Trouvé:	8.53;	19.65;	7.56[1])

Acide aminé tiré du sel cuivrique: 0 gr,1431 correspondait à 15 cc,10 d'hyposulfite N/14,01 (10,55 % N).

II b. 0 gr,3625 perdit dans le vide à 110° 0 gr,0021; 0 gr,1400 correspondait à 11 cc,99 d'hyposulfite N/14,01 (8,57 % N); 0 gr,1923 donna 0 gr,0472 CuO (19,60 % Cu).

La différence que I b présente au point de vue de la teneur en eau de cristallisation, tient probablement à ce qu'une partie du sel anhydre II b aurait cristallisée en même temps que I b.

---

[1]) Calculé sur la matière hydratée.

La méthode que nous venons d'exposer pour la préparation de la γ-oxyproline fournit un rendement de 33 pour cent de dérivé benzoylé calculé sur la matière première, rendement sensiblement supérieur à celui obtenu par la méthode Leuchs. En dehors de cet avantage, notre procédé se distingue par une plus grande simplicité d'exécution.

Le filtrat cuivreux provenant du dérivé cuivrique de la γ-oxybenzoylproline (CuF, p. 249) fut débarrassé de cuivre au moyen de l'hydrogène sulfuré, et ensuite on enleva ce dernier à l'aide d'un courant rapide d'air. Le liquide fut étendu à 1 litre.

Dans le dosage d'azote d'après Kjeldahl, 10 cc. de ce liquide correspondaient à $3^{cc},95$ d'hyposulfite N/14,01, de sorte que la teneur totale en azote s'élevait à 395 mgr. Titrés au formol, 10 cc. ne demandèrent que $0^{cc},5$ de solution N/5 de NaOH, ce qui pour la totalité du liquide correspondait à 140 mgr. de N titrable de cette façon, valeur qui cependant est trop sujette à caution pour légimiter aucune conclusion au delà de celle-ci, qu'il ne reste pas beaucoup d'azote titrable au formol.

Le restant, qui se montait à 980 cc., fut rapproché dans le vide vers 100 cc., et ce résidu fut traité par l'acide sulfurique, suivant le procédé décrit plus haut, dans le but d'obtenir l'α-benzoylamino-γ-δ-dioxyvalérolactone. Le premier traitement fournit 1 gr., le second $0^{gr},45$. Les deux préparations furent recristallisées conjointement dans de l'eau, et les cristaux ainsi obtenus subirent la dessiccation dans le vide sulfurique.

$0^{gr},1037$ correspondait à $6^{cc},17$ d'hyposulfite N/14,01; $0^{gr},2112$ titré à chaud au moyen d'une solution normale au 1/5 de NaOH, avec la phénolphtaléine comme indicateur, en demanda $4^{cc},49$.

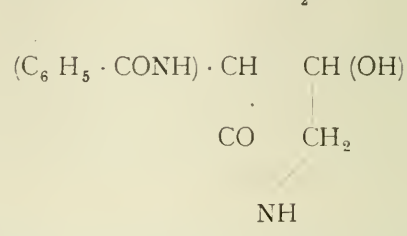
Calculé:	N 5,96;	alcali: $4^{cc},49$
Trouvé:	5,95;	$4^{cc},49$

Des 1464 mgr. d'azote faisant partie de la matière première (B_{II}) ont ainsi été récupérés: à l'état de γ-oxybenzoylproline $483^{mgr},7$, sous forme de benzoyldioxylactone $86^{mgr},4$, ce qui fait un total de 570 mgr. d'azote, soit 39 pour cent de l'azote de la matière première.

γ-oxyornithine.

$CH_2(NH_2) \cdot CH(OH) \cdot CH_2 \cdot CH(NH_2) \cdot COOH$.

1. α-benzoylamino-γ-oxypipéridone.



Dans un matras de 3 litres de capacité, fermé avec un bouchon perforé, et muni, durant tout le cours des opérations que nous allons relater, d'un tube à chlorure de calcium, on fit dissoudre — incomplètement — dans un litre d'alcool absolu 38gr,6 d'α-benzoylamino-δ-brome-γ-oxyvalérolactone, dont, après refroidissement, on vit se déposer une partie dans la solution obtenue, à laquelle, ayant plongé le matras dans de l'eau glacée, on fit arriver alors pendant cinq heures un courant rapide d'ammoniaque gazeuse, obtenue en faisant tomber goutte à goutte une solution concentrée d'ammoniaque sur de la potasse solide et ayant soin de dessécher l'ammoniaque à l'aide de chaux sodée et de chlorure de calcium.

Ensuite, en discontinuant le courant d'ammoniaque, on retira le matras de l'eau glacée pour l'abandonner à la température de la chambre, en prenant soin que l'air ne pût pénétrer dans le matras que par le tube à chlorure de calcium. Le jour d'après, un nouveau courant d'ammoniaque, maintenu pendant une heure, acheva de dissoudre le composé bromique; cependant, il s'était formé quelques cristaux, qui par leur aspect granuleux étaient nettement dissemblables à ceux du corps bromé, qui présentaient une forme lamellée. Après avoir laissé le matras encore quatre jours à la température ambiante, on constata, par un titrage alcalimétrique d'une petite prise, que la liqueur était 5-normale relativement à l'ammoniaque.

La proportion de brome titrable d'après Volhard, se trouva correspondre à 130 cc. de brome normal, la valeur théorique étant de 129cc,5.

Séparés par décantation du liquide, lavés à la trompe à 3 fois 10 cc. d'alcool absolu, enfin séchés à l'air, les cristaux (I)

accusèrent un poids de 8gr,8. On réunit l'alcool de lavage à la liqueur principale, puis fit passer par distillation dans le vide (20 mm.) dans un récipient chargé d'acide sulfurique, en chauffant au bain-marie (à 40°) afin d'accélérer la distillation de l'alcool et de l'ammoniaque. Lorsque le volume du liquide eut été réduit vers 700 cc., on vit commencer une nouvelle cristallisation, accompagnée de soubresauts très forts. On interrompit alors la distillation, pour essorer ensuite les cristaux à la trompe. Lavés à 3 fois 10 cc. d'alcool et séchés à l'air, ils pesaient 5gr,9 (II). Après avoir soumis la liqueur filtrée et l'alcool de lavage à une nouvelle évaporation dans le vide, évaporation qui en réduisit le volume à 300 cc. environ, on en sépara par filtration une seconde portion de cristaux, dont, après lavage à 3 fois 5 cc. d'alcool et dessiccation à l'air, le poids était de 3gr,6 (III). On évapora enfin dans le vide l'eau-mère et l'alcool de lavage à siccité, puis, à l'aide de 3 fois 10 + 4 fois 5 cc. d'eau glacée, on transféra le résidu solide dans un gobelet, qu'on abandonna deux heures dans l'eau glacée, pour en essorer ensuite le contenu à la trompe. Les cristaux laissés sur le filtre furent lavés à 7 fois 10 cc. d'eau glacée. La dernière eau de lavage additionnée de nitrate d'argent en solution nitrique, ne montra qu'une opalescence extrêmement faible. Le poids des cristaux séchés à l'air (IV) était de 5gr,2. Le rendement total en «pipéridone» se montait ainsi à 8,8 + 5,9 + 3,6 + 5,2 = 23gr,5, soit 73 pour cent de la quantité théorique.

L'eau-mère provenant de IV n'a pas été examinée de plus près. Elle ne contient pas de «pipéridone», mais bien une substance acide, qui ne renferme pas d'azote titrable au formol et ne donne pas de sel cuivrique.

Pour faire subir à la «pipéridone» une nouvelle cristallisation, on fit dissoudre les 23gr,5 obtenus dans 200 + 150 + 75 + 30 + 20 cc. d'eau bouillante. Le lendemain, on essora à la trompe et lava à 3 fois 5 cc. d'eau glacée, ce qui fournit 18 gr. (A). En évaporant l'eau-mère à la moitié de son volume, on récupéra encore 4 gr. (B).

On préleva sur A un échantillon pour en faire l'analyse après dessiccation dans le vide sulfurique.

0gr,0969 correspondait dans le dosage d'après Kjeldahl à 11cc,52 d'hyposulfite normal au 1/14,01; 0gr,1211 à 14cc,47 de cette même solution.

Calculé... 11,97 % N
Trouvé... 11,89—11.95 % N

La «pipéridone» fond au «bloc Maquenne» (5 secondes) à 225—226°.

Elle est assez difficilement soluble dans l'eau chaude, difficilement soluble dans l'eau froide; dans l'alcool elle ne se dissout guère, dans le chloroforme et l'éther pas du tout. L'alcool la fait cristalliser sous forme d'aiguilles rectangulaires de longueur variable.

La solution aqueuse a une réaction neutre, réaction à laquelle l'addition de formol ne change rien. Le traitement par l'eau de baryte a pour effet de rompre la liaison CO · NH de la «pipéridone» sous formation de l'*α*-benzoyle-*γ*-oxyornithine.

2. *α*-monobenzoyle-*γ*-oxyornithine.

$CH_2\,(NH_2)\cdot CH\,(OH)\cdot CH_2\cdot CH\,(NH\cdot CO\cdot C_6H_5)\,COOH.$

10 grammes de la «pipéridone» recristallisée furent délayés dans 200 cc. d'eau de baryte saturée à froid (2 équivalents) et chauffés durant une heure dans un matras au bain d'eau bouillante, puis on abandonna le mélange pendant deux jours, en agitant fréquemment. La totalité de la «pipéridone» était alors entrée en dissolution, et l'on amena le liquide au volume de 250 cc.

5 cc., titrés au formol, demandèrent $4^{cc},27$ de NaOH normal au $^1/_5$, correspondant à $11^{mgr},96$ d'azote, soit, en tout, 598 mgr. d'azote titrable au formol, tandis que le calcul en donnerait $598^{mgr},5$, si la totalité de la «pipéridone» avait été transformée en monobenzoylornithine.

La portion principale fut débarrassée de baryte à l'aide d'acide sulfurique, et le sulfate de baryte lavé soigneusement à l'eau chaude. (Le précipité contenait alors $3^{mgr},7$ d'azote). Le liquide filtré réuni à l'eau de lavage fut rapproché dans le vide jusque vers 20 cc. Dans le matras encore chaud, on vit déjà se produire une cristallisation abondante. On versa le magma (I) dans un gobelet, et l'on fit passer le restant (II), à l'aide de 50 cc. d'alcool à 93 pour cent, dans un autre gobelet. Après avoir séjourné dans la glacière jusqu'au lendemain, I fut essoré à la trompe, et les cristaux lavés à 2 fois 5 cc. d'alcool à 93 pour cent et séchés à l'air; ils pesaient alors $7^{gr},5$. La liqueur filtrée et l'eau de lavage réunies à II furent évaporées à sec dans le vide, et le

résidu — à l'aide d'une vingtaine de cc. d'alcool à 93 pour cent — introduit dans un gobelet, qu'on abandonna dans la glacière jusqu'au jour suivant, pour essorer alors à la trompe et laver à 2 fois 25 cc. d'alcool à 93 pour cent. Poids après dessiccation à l'air· 3 gr. (C). Rendement: 7,5 + 3,0 = 10gr,5, soit 97,5 pour cent du calcul. — I fut dissous, à chaud, dans 70 cc. d'eau, puis additionné de 140 cc. d'alcool à 93 pour cent. Le lendemain, on essora à la trompe, lava à 3 fois 5 cc. d'alcool à 50 pour cent, et sécha à l'air; poids: 4gr,5 (A). Enfin, on évapora à sec dans le vide le liquide filtré et l'alcool de lavage (finalement dans le vide sulfurique à la température ordinaire), et l'on récupéra ainsi 3gr,0 (B).

A. 1gr,4364 perdit dans le vide à 70° 0gr,0076 (le premier jour 0gr,0065, le deuxième 0gr,0011, le troisième plus rien). Il ne paraît donc pas y avoir eu formation appréciable de lactone.

0gr,1056 (matière anhydre) donna 0gr,0614 H_2O et 0gr,2212 CO_2.

0gr,1048 correspondait dans le dosage d'azote d'après Kjeldahl à 11cc,10 d'hyposulfite N/14,01.

0gr,1808, titré au formol, demanda 3cc,36 de NaOH N/5.

		Calculé	Trouvé	Valeurs calculées pour l'α-monobenzoylornithine		
C_{12}	144	57,11	57,13	C_{12}	144	60,97
H_{16}	16,12	6,39	6,50	H_{16}	16,12	6,83
O_4	64	25,38	—	O_3	48	20,33
N_2	28,02	11,12	11,06	N_2	28,02	11,87
	252,14	100,00			236,14	100,00
N titrable au formol		5,56	5,30			

B. 0gr,1073 correspondait à 11cc,91 d'hyposulfite N/14,01.
0gr,2101 demandait 4cc,00 de NaOH N/5 dans la titration au formol.

C. 0gr,1987 correspondait à 21cc,96 d'hyposulfite N/14,01.
0gr,3002 demandait 5cc,73 de NaOH N/5 dans la titration au formol.

Trouvé	B	C
N.....	11,10	11,05
N titrable au formol	5,33	5,35

Les préparations étaient toutes d'une couleur parfaitement blanche.

L'α-monobenzoyle-γ-oxyornithine fond au «bloc Maquenne» (5 secondes) à 255—256°. Une détermination tout à fait corres-

pondante faite sur l'α-monobenzoylornithine (synthétique) donna 264—267° (Sørensen).

L'α-monobenzoyle-γ-oxyornithine est facilement soluble dans l'eau, même à la température ordinaire, et ne s'en laisse recristalliser sans une grande perte; dans l'alcool absolu elle se dissout à peine (l'alcool à 50 pour cent est le meilleur milieu de recristallisation). Elle cristallise de l'eau sous forme d'aiguilles prismatiques bien développées.

Comme on devait s'y attendre, l'α-monobenzoyle-γ-oxyornithine est plus soluble dans l'eau que ne l'est l'α-monobenzoylornithine[1]). Au sujet de celle-ci, Sørensen fait observer ce qui suit: «Ce composé est difficilement soluble dans l'eau froide et un peu plus soluble dans l'eau bouillante . . . . . et il ne possède point cette disposition marquée à la cristallisation qui caractérise ce dernier (le dérivé benzoylé en δ).»

3. Monopicrate de γ-oxyornithine.

$$C_5 H_{12} O_3 N_2 \cdot C_6 H_3 O_7 N_3 + H_2 O.$$

On chauffa 5 grammes de «pipéridone» en présence de 50 cc. d'acide chlorhydrique concentré pendant 7 heures dans un ballon au bain-marie bouillant. La solution limpide fut ensuite étendue avec un égal volume d'eau, et l'acide benzoïque extrait à l'éther. Les extraits éthérés, soumis à l'évaporation, donnèrent le poids calculé d'acide benzoïque. D'après des dosages réalisés sur des petites prises, la teneur totale en azote se montait à 575 mgr,6, celle en azote titrable au formol à 575 mgr. On débarrassa alors, par distillation dans le vide, la solution principale d'une grande partie de l'acide chlorhydrique, puis le reste fut enlevé par addition à la température ambiante de carbonate d'argent, et enfin, un excès d'argent fut éliminé à l'aide d'acide chlorhydrique, laissant une liqueur à réaction faiblement alcaline. En l'évaporant maintenant dans le vide vers 20 cc., on aperçut un très vif dégagement de gaz (CO_2), la réaction devenant fortement alcaline au tournesol. On fit arriver un courant d'acide carbonique jusqu'à réaction neutre, puis on ajouta une solution alcoolique concentrée d'acide picrique en quantité calculée pour un dipicrate. Dans le commencement la liqueur demeura limpide; mais le lendemain

[1]) Sørensen: Ces mêmes Comptes-rendus, **10**, 125 (1911).

une certaine quantité de cristaux s'étaient formés, qu'on essora à la trompe et lava d'abord à 3 fois 5 cc. d'eau, puis à l'alcool jusqu'à ce que celui-ci s'en écoulât incolore. Poids après dessiccation à l'air: $3^{gr},5$.

La liqueur filtrée, évaporée dans le vide, laissa une huile qui, triturée à l'alcool, fournit une certaine quantité ($0^{gr},7$) de picrate manifestement impur, qu'on ne soumit pas à l'analyse. Les $3^{gr},5$ des cristaux furent recristallisés dans une faible portion d'eau, essorés et lavés à 3 fois 5 cc. d'eau, puis à l'alcool et enfin à l'éther. Séchés à l'air ils pesaient $2^{gr},6$. La préparation servit directement à l'analyse.

$1^{gr},0374$ perdit dans le vide à 75^0 $0^{gr},0466$.

$0^{gr},6573$ de matière déshydratée fut dissous dans 100 cc. d'eau chaude additionnée de 10 cc. d'acide sulfurique à 10 %, après quoi l'acide picrique fut extrait à l'éther. Les extraits éthérés subirent des lavages avec des petites portions d'eau, auxquelles on enleva ensuite l'acide picrique au moyen d'éther. Les extraits éthérés réunis laissèrent par évaporation $0^{gr},4070$.

Les solutions aqueuses furent réunies, complétées à 200 cc. et divisées en deux parties égales. Chacune de ces parties fut évaporée à sec dans un ballon de Kjeldahl, et le contenu d'azote des deux résidus fut établi. Chaque portion se trouva correspondre à $24^{cc},32$ d'hyposulfite N/14,01.

Calculé:	N 7,41,	Ac. picrique 60,57,	H_2O[1]) 4,55
Trouvé:	7,40,	61,93,	4,49

Les valeurs y correspondant calculées pour le monopicrate d'ornithine sont:

N 7,74, Acide picrique 63,4.

Le monopicrate de γ-oxyornithine fond au «bloc Maquenne» en 5 secondes à 185—190°, en se décomposant. Ce composé se dissout difficilement dans l'eau à froid, plus facilement à chaud, tandis que ni l'alcool ni l'éther ne le dissolvent. Dans l'eau il cristallise sous la forme d'une poudre d'un jaune clair éclatant. Examinée au microscope, cette poudre se montre formée d'aiguilles rectangulaires courtes et épaisses, le plus souvent groupées en étoiles.

A titre de comparaison, nous pouvons citer que, d'après Fischer et Raske[2]), Riesser et, enfin, Kossel et Weiss[3]),

[1]) Calculé sur matière hydratée.

[2]) Fischer et Raske: Berichte der d. chem. Ges., **38**, 3607 (1905).

[3]) Kossel et Weiss: Zeitschrift für physiol. Chemie, **68**, 161 (1910).

en ajoutant de l'acide picrique alcoolique à une solution de carbonate d'ornithine, on obtient un dipicrate, même quand la proportion dudit acide est moindre que celle calculée pour un monopicrate. Au sujet du monopicrate préparé par Kossel et Weiss, ces savants se prononcent comme suit: «Nach Riesser krystallisiert dieses Salz zuweilen mit $1^1/_2$ Mol. Wasser; die von uns dargestellten Krystalle enthielten kein Krystallwasser. Der Schmelzpunkt lag in Übereinstimmung mit E. Fischer und Raske sowie mit Riesser unscharf bei 170°.»

4. Chloroplatinate du γ-oxyornithine.

$(C_5 H_{11} O_3 N_2)_2 Pt Cl_6 + 1^1/_2 H_2O$ (soit 1 H_2O).

On prépara, en opérant de la manière décrite plus haut, une solution de γ-oxyornithine carbonique, à laquelle on ajouta une solution à 20 pour cent de chlorure de platine en proportion calculée pour le di-chloroplatinate. Comme le mélange demeura limpide, on l'additionna d'un grand excès d'alcool, qui ne tarda pas à faire cristalliser le chloroplatinate, lequel présentait une couleur jaunâtre. Le lendemain on essora à la trompe, puis fit dissoudre le sel dans un peu d'eau, pour le précipiter de nouveau au moyen d'alcool. Avant d'analyser la préparation, on la sécha à l'air.

$0^{gr},4611$ perdit dans le vide à 102° — en prenant une couleur assez foncée — $0^{gr},0142$; $0^{gr},2100$, fondu en présence de soude et de salpêtre, donna $0^{gr},0569$ de Pt et $0^{gr},0619$ de HCl (d'après Volhard).

Calculé[1]): Pt 27,80; HCl 30,21; H_2O 3,69 ($1^1/_2$ mol.), ou 2,50 (1 mol.).
Trouvé[1]): 27,96; 30,40; 3,18.

A l'inverse de la combinaison correspondante de l'ornithine, le chloroplatinate de γ-oxyornithine cristallise facilement. Les cristaux affectent la forme de petites aiguilles courtes et épaisses, rassemblées en faisceaux. Chauffé à 100°, le composé subit une décomposition partielle, mais bien nette, en se fonçant, et l'observation au microscope montra que cette préparation était constituée en majeure partie par de jolis cristaux en forme de sapin.

En terminant cet exposé, je me fais un devoir de présenter encore ici à M. le Professeur S. P. L. Sörensen mes remerciements bien sincères du gracieux concours qu'il n'a cessé de me prodiguer au cours de mon stage au Laboratoire de Carlsberg.

[1]) Sur matière anhydre.

ÉTUDE SUR LA CINÉTIQUE D'UNE HYDROLYSE ENZYMATIQUE DE LA GLYCYLGLYCOCOLLE.

PAR

KARL GUSTAV DERNBY.

La marche des scissions protéolytiques a, pendant cette dernière dixaine d'années, fait l'objet d'un grand nombre de recherches des plus actives. On s'est notamment demandé si ces réactions enzymatiques obéissent aux mêmes lois très simples qui régissent celles où des catalyseurs d'ordre purement chimique entrent en jeu, ou bien si elles suivent une cinétique toute spéciale.

Parmi les savants qui se sont occupés tout particulièrement des scissions enzymatiques des dipeptides, il convient de citer en premier lieu Abderhalden et ses collaborateurs et, d'autre part, Euler. Abderhalden et Koelker[1]), Abderhalden et Michaëlis[2]), Abderhalden et Brahm[3]), opérant au moyen de diverses enzymes protéolytiques le dédoublement de plusieurs dipeptides optiquement actives, ont déterminé la vitesse de la réaction en mesurant les variations que subit le pouvoir rotatoire de la solution expérimentée. A la suite de leurs recherches, ils ont établi que, dans la plupart des cas, la réaction marche plus vite qu'elle ne ferait, si elle se conformait à la formule monomoléculaire, et Michaëlis a cru pouvoir l'exprimer par une formule empirique:

$$k = \ln \frac{a}{a - x} + \varepsilon \cdot \frac{x}{t}$$

Euler[4]), de son côté, a réalisé la scission de la glycylglycocolle et de plusieurs autres dipeptides en solution alcaline et à l'aide de diverses préparations d'érepsine, et il a établi la vi-

[1]) Abderhalden et Koelker: Zeitschr. für physiol. Chemie, **51**, 294 (1907).
[2]) Abderhalden et Michaëlis: Ibidem, **52**, 326 (1907).
[3]) Abderhalden et Brahm: Ibidem, **57**, 342 (1908).
[4]) Euler: Arkiv för Kemi, Mineralogi och Geologi, N° 31 et 39. Stockholm 1907.

tesse de la réaction en mesurant la modification subie par la conductivité électrique. Il conclut de ses recherches que la constante de réaction monomoléculaire va en diminuant vers la fin de la réaction.

Dans l'exposé qu'on va lire, je me propose de rechercher comment il s'est fait qu'on soit arrivé à des résultats aussi contradictoires.

On comprend facilement que les choses se compliquent singulièrement lorsqu'il s'agit du dédoublement des substances protéiques naturelles, telles que caséine, peptone etc. En règle générale, on a constaté que la réaction prend une marche plus lente qu'elle ne ferait, si elle avait suivi la formule monomoléculaire. C'est notamment ce qui a été trouvé par V. Henri et Larguier des Bancels[1]) dans le cas de la scission trypsique de la gélatine et de la caséine, ainsi que par Sjöquist[2]) en ce qui concerne le dédoublement de l'albumine par la pepsine.

Dans certains cas spéciaux, on est parvenu à établir des formules empiriques pour la protéolyse. A ce sujet, nous pouvons nous borner à rappeler la règle de Schütz, de laquelle Arrhenius a déduit sa formule bien connue[3]), et l'auteur de la présente étude a montré que la marche que prend la réaction dans l'autolyse de la levure peut être représentée par une formule empirique, à savoir:

$$k = \frac{1}{\sqrt{t}} \ln \frac{a + x}{a - x}$$ [4])

Quant aux causes qui font diminuer la vitesse des scissions protéolytiques plus vite qu'on ne devait s'y attendre d'après la formule

$$\frac{dx}{dt} = k\,(a - x),$$

quelques chimistes les ont cherchées dans l'influence exercée par les produits de la réaction, tandis que d'autres, et parmi ceux-ci Herzog[5]), estiment que cela tient plutôt aux conditions de viscosité.

Des recherches plus récentes ont mis en évidence ce fait que, pour expliquer les phénomènes qui nous occupent, il est

[1]) Comptes rendus de la Société de Biologie, **55**, 563 (1903).
[2]) Skandinavisches Archiv für Physiologie, V. (1895).
[3]) Meddelanden från K. Vet. Ak. Nobelinstitut, **1**, 9. Stockholm (1908).
[4]) Zeitschrift für physiologisehe Chemie, **89**, 425 (1914).
[5]) Ibidem, **41**, 416 (1904).

indispensable de tenir compte des modifications qu'éprouve la concentration en ions hydrogène pendant le cours de la réaction. En effet, les travaux effectués dans ce domaine par S. P. L. Sörensens[1]) et, après lui, par Michaëlis[2]) et d'autres, ont eu pour conséquence de changer radicalement la conception de la structure des enzymes et de leur mode d'action, en ce sens qu'il faut les considérer comme des électrolytes amphotères à molécules très grandes, dont le degré de dissociation est une simple fonction de la concentration en ions hydrogène du milieu, et dont la partie catalytiquement active est constituée ou bien par la molécule non-dissociée, ou par un des ions de la molécule dissociée. Dans le cas des enzymes protéolytiques comme la pepsine et la trypsine, c'est l'anion qu'on a reconnu être doué du pouvoir actif.

D'après Euler, la constante de dissociation, K_a, de la glycylglycocolle considérée comme acide est supérieure à celle de la glycocolle produite par la réaction. Il s'ensuit que, pour une même teneur en hydroxyde sodique, le liquide expérimenté deviendra de plus en plus alcalin à mesure que la durée de la réaction se prolonge.

On trouvera ci-dessous l'exposé des recherches que, sur les conseils de M. le Professeur S. P. L. Sörensen, j'ai entreprises dans le but de déterminer les modifications que subit la concentration en ions hydrogène au cours d'une scission de la glycylglycocolle opérée au moyen de l'enzyme contenue dans le jus de levure pressée et qui a de l'analogie avec l'érepsine. Nous examinerons ensuite la question relative à l'influence exercée par ces modifications sur la vitesse de la réaction, et enfin nous chercherons à élucider l'allure que prend la réaction lorsque la concentration ionique est maintenue constante.

A. Les Constantes de Dissociation de la glycylglycocolle et de la glycocolle.

Grâce aux recherches de Winkelblech[3]), de Walker[4]) et de Lundén[5]), les conditions de dissociation des électrolytes am-

[1]) Ces mêmes Comptes-rendus, **8**, 1 (1908).
[2]) Voir: Michaëlis: Die Wasserstoffionenkonzentration. Berlin 1914.
[3]) Zeitschr. für physikal. Chemie, **36**, 546 (1901).
[4]) Ibid., **49**, 82 (1904), et **51**, 706 (1905).
[5]) Medd. fr. K. Vet. Ak. Nobelinstitut, **1**, 11 (Stockholm 1908).

photères se trouvent maintenant complètement élucidées. C'est ainsi qu'il a été établi que l'addition d'un acide donne à la glycocolle la réaction d'une base, et que mélangé avec un hydrate d'alcali ce corps se comporte, au contraire, comme un acide. Au moyen de la conductibilité et du degré d'hydrolyse, les auteurs cités ont pu calculer la constante de dissociation acide et la constante de dissociation basique — désignées respectivement par K_a et K_b — d'un grand nombre d'électrolytes amphotères.

Dans le but de vérifier les valeurs déterminées d'avance des constantes de dissociation de la glycylglycocolle et de la glycocolle — valeurs qui présentaient un certain intérêt pour les recherches que nous allons relater —, j'ai procédé à des déterminations de ces constantes suivant une méthode différente de celle employée par mes devanciers: au lieu de mesurer la conductibilité, je me suis appliqué à déterminer la concentration des ions hydrogène de solutions des deux corps additionnées de différentes quantités, respectivement, d'hydroxyde de sodium et d'acide chlorhydrique.

A moins d'indication contraire, ces essais ont été réalisés de la manière suivante:

Des fioles jaugées de la capacité de 20 cc., au préalable remplis d'air atmosphérique débarrassé de son acide carbonique, furent chargées chacune de 10 cc. d'une solution 0,8 normale de glycocolle ou 0,4 normale de glycylglycocolle et, de plus, de doses variées d'acide chlorhydrique ou d'hydroxyde sodique dépourvu d'acide carbonique. Complétées jusqu'au trait avec de l'eau exempte d'acide carbonique, les solutions de glycocolle étaient donc toujours 0,4 normales, et celles de glycylglycocolle 0,2 normales.

Dans environ 5 cc. du mélange on détermina alors la concentration en ions hydrogène (représentée dans les tableaux par l'exposant des ions hydrogène $p_H{}^+$) à l'aide d'un appareil Hasselbalch traversé par un courant d'hydrogène. Le dispositif était exactement le même que celui indiqué par S. P. L. Sörensen dans ses «Etudes enzymatiques II»[1]). Toutes les mesures furent faites à une température comprise entre $17^0{,}5$ et $18^0{,}5$, et ont été réduites à 18^0.

Une fois que l'on connaît la concentration en ions hydrogène,

[1]) Ces mêmes Comptes-rendus, **8**, 1 (1909).

il est facile de calculer la constante de dissociation, K_a ou K_b. Soit a la concentration primitive de la glycocolle, et b celle de l'hydroxyde sodique, on aura — supposant que la glycocolle sodique formée ait subi une dissociation complète, et que, ce qui n'est pas tout à fait correct, la concentration de la glycocolle dissociée puisse être posée égale à celle de l'hydroxyde sodique b, — l'équation suivante:

$$\frac{[\overset{+}{H}] \cdot [\text{glyc. diss.}]^{\div}}{[\text{glyc. non diss.}]} = K_a \quad \text{ou} \quad \frac{[\overset{+}{H}] \cdot b}{a \div b} = K_a$$

De la même manière, à supposer que dans ce cas-ci la concentration de l'acide chlorhydrique soit égale à b, on aura pour l'hydrochlorure de glycocolle:

$$\frac{[\overset{\div}{OH}] \cdot [\text{glyc. diss.}]^{+}}{[\text{glyc. non diss.}]} = K_b,$$

où, désignant par K_e la constante de dissociation de l'eau,

$$[OH]^{\div} = \frac{K_e}{[H]^+},$$

de sorte que l'équation relative à l'hydrochlorure de glycocolle pourrait en définitive être formulée ainsi:

$$\frac{\frac{K_e}{[H]^+} \cdot b}{a \div b} = K_b.$$

Cependant, comme nous venons de le faire observer, il n'est pas parfaitement exact d'admettre que la totalité du sel de glycocolle ait été dissociée. Si nous posons le degré de dissociation égal à α, la concentration en ions glycocolle sera $\alpha \cdot b$, et les équations indiquées ci-dessus devront être modifiées comme suit:

$$K = \frac{[\overset{+}{H}] \cdot \alpha \cdot b}{a \div b} \quad \text{et} \quad K = \frac{\frac{K_e}{[H]^+} \cdot \alpha \cdot b}{a \div b}.$$

Comme il n'existe pas de déterminations de α pour les sels de glycocolle ou de glycylglycocolle, nous employerons dans la suite, dans les calculs de K_a, α de l'acétate de sodium, et, dans ceux de K_b, α du chlorure d'ammonium, puisque ce sont là les cas qui y correspondent le mieux. Nous avons calculé α au moyen de la formule $\alpha = \frac{\lambda_v}{\lambda_\infty}$ dans les Tables de Kohlrausch[1]).

[1]) Kohlrausch: Ann. d. Phys. und Chemie, **66**, 785 (1898); Sitz. Ber. d. K. Preuss. Akademie d. Wissensch., **26**, 581 (1902); Kohlrausch und Holborn: Das Leitvermögen der Elektrolyte (1898).

Les constantes de dissociation consignées aux tableaux ci-dessous ont été établies, soit sur la base des valeurs de α données dans les tableaux même, soit en supposant que $\alpha = 1$.

1 Glycocolle.

TABLEAU 1a.

K_a à la température de 18°.

Concentration de la glycocolle (a) $= 40 \times 10^{-2}$.

No	[NaOH] b $\times 10^2$	$p_H{}^+$	α pour NaAc	$K_a \times 10^{10}$		
				$\alpha = \frac{\lambda_v}{\lambda_\infty}$	$\alpha = 1$	$\alpha = \frac{\lambda_v}{\lambda_\infty} \cdot \eta^c$
1	1	8,345	0,91	1,06	1,16	1,06
2	2	8,635	0,88	1,05	1,19	1,06
3	3	8,822	0,86	1,05	1,22	1,06
4	4	8,956	0,83	1,03	1,23	1,05
5	6	9,156		1,01	1,23	1,03
6	8	9,316	—	0,98	1,21	1,01
7	10	9,426	0,79	0,99	1,25	1,03
8	15	9,676	—	0,98	1,27	1,04
9	20	9,877	0,74	0,99	1,34	1,06
10	25	10,095	—	0,97	1,34	1,05
11	30	10,337	0,70	0,97	1,38	1,07
12	35	10,709	—	0,90	1,37	1,02

TABLEAU 1b.[1])

K_a à 18°.

Conc. de glyc. + Concentr. de NaOH $= 10 \times 10^{-2}$

Concentration de $\overset{+}{[Na]}$ partout $= 10 \times 10^{-2}$.

No	Composition du mélange			$p_H{}^+$	$K_a \times 10^{10}$	
	[glyc.] a $\times 10^2$	[NaOH] b $\times 10^2$	a ÷ b $\times 10^2$		$\alpha = 0.79$	$\alpha = 1$
1	9,75	0,25	9,5	8,237	1,21	1,53
2	9,5	0,5	9,0	8,575	1,17	1,48
3	9,0	1,0	8,0	8,929	1,16	1,47
4	8,0	2,0	6,0	9,364	1,14	1,44
5	7,0	3,0	4,0	9,714	1,15	1,45
6	6,0	4,0	2,0	10,140	1,15	1,45
7	5,5	4,5	1,0	10,482	1,17	1,48

[1]) Les calculs sont basés sur les mesures faites dans un autre but par M. S. Palitzsch, voir ces mêmes Comptes-rendus, **8**, 41, Tab. 3 (1909).

TABLEAU 2.[1]

K_b à 18°. Concentration de glycocolle (a) = 40 × 10^{-2}.

No	[HCl] b × 10^2	p_H+	α pour NH_4Cl	$K_b \times 10^{12}$	
				$\alpha = \frac{\lambda_v}{\lambda_\infty}$	$\alpha = 1$
1	1	3,993	0,94	1,71	1,82
2	2	3,676	0,92	1,68	1,82
3	3	3,493	0,91	1,65	1,81
4	4	3,358	0,90	1,66	1,84
5	5	3,257	0,89	1,64	1,84
6	6	3,164	0,88	1,62	1,84
7	8	3,007	0,86	1,59	1,85
8	10	2,884	0,85	1,56	1,83
9	20	2,403	0,82	1,60	1,95
10	30	1,967	0,82	1,59	1,99

2. Glycylglycocolle.

TABLEAU 3.

K_a à 18°. Concentration de glycylglycocolle (a) = 20 × 10^{-2}.

No	[NaOH] b × 10^2	p_H+	α pour NaAc	$K_a \times 10^9$		
				$\alpha = \frac{\lambda_v}{\lambda_\infty}$	$\alpha = 1$	$\alpha = \frac{\lambda_v}{\lambda_\infty} \cdot \eta^c$
1	2	7,478	0,88	3,3	3,70	3,3
2	4	7,813	0,85	3,3	3,84	3,3
3	6	8,054	0,82	3,1	3,79	3,2
4	8	8,240	0,80	3,1	3,84	3,2
5	10	8,398	0,79	3,1	3,90	3,2

TABLEAU 4.

K_b à 18°. Concentration de glycylglycocolle (a) = 20 × 10^{-3}.

No	[HCl] b × 10^2	p_H+	α pour NH_4Cl	$K_b \times 10^{11}$	
				$\alpha = \frac{\lambda_v}{\lambda_\infty}$	$\alpha = 1$
1	2	4,119	0,92	0,95	1,04
2	4	3,763	0,90	0,95	1,05
3	6	3,534	0,88	0,93	1,06
4	8	3,333	0,86	0,90	1,04
5	10	3,152	0,85	0,88	1,03

[1]) V. la note au bas de la page précédente.

L'examen de l'avant-dernière colonne du Tableau 1 a ($\alpha = 1$) semble montrer que les valeurs de K_a vont en augmentant graduellement avec la concentration de l'hydroxyde de sodium; c'est à quoi on devait s'y attendre pour autant que α est supposé être égal à 1. Au contraire, si l'on tient compte des valeurs variables de α, on constate que K_a va en diminuant quand la concentration dudit hydroxyde s'accroît. Cependant, au Tableau 1 b, où la concentration en ions sodium est partout la même et où, par conséquent, α a aussi une valeur constante, on voit que la constante de dissociation ne baisse ni ne monte. En somme, il paraît donc que le degré de dissociation arbitrairement choisi, α, ne cadre point avec la réalité.

Il ressort du Tableau 3 qu'il en est de même pour K_a de la glycylglycocolle, et les Tableaux 2 et 4 font voir que la même remarque s'applique, bien qu'à un degré moindre, au K_b de la glycocolle et de la glycylglycocolle. Or, il ne paraît guère possible que les sels de glycocolle et de glycylglycocolle soient plus dissociés que l'acétate de sodium et le chlorure d'ammonium.

Obéissant à un conseil que m'a donné M. le Professeur Arrhenius, j'ai appliqué une correction relative à la viscosité des solutions expérimentées, en la supposant égale à celle d'une solution d'acétate de soude. Les chiffres sont tirés d'un mémoire de R. Reiher[1]), et α a été calculé d'après la formule $\alpha = \frac{\lambda_v}{\lambda_\infty} \cdot \eta^c$, où η représente le coefficient de viscosité de la solution 1-normale de la substance considérée, à supposer que celui de l'eau soit posé égal à 1; c signifie la concentration.

Dans la dernière colonne des Tableaux 1a et 3 sont inscrites les valeurs de K_a corrigées, ainsi que je viens de l'indiquer, au point de vue de la viscosité, correction qui, on le voit, donne des valeurs beaucoup plus constantes de K_a pour les différentes concentrations d'hydroxyde sodique.

Le Tableau 5 ci-après contient les valeurs de K_a et K_b les plus vraisemblables que nous ayons trouvées. A titre de comparaison, nous y ajoutons celles établies antérieurement par Euler et Winkelblech[2]).

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[1]) Zeitschr. für physik. Chemie. **2**, 750 (1888).

[2]) Cité d'après Lundén: Medd. fr. K. Vet. Ak. Nobelinstitut **1**, 11, Stockholm (1908).

TABLEAU 5.

K_a et K_b	Valeurs trouvées			
	par nous-même		par nos prédécesseurs[1])	
	à 18°	donc, à 25°	à 25°	Expérimentateur
K_a de glycocolle	$1{,}05 \times 10^{-10}$	—	$1{,}8 \times 10^{-10}$	Winkelblech
K_b » »	$1{,}7 \times 10^{-12}$	$2{,}7 \times 10^{-12}$	$2{,}7 \times 10^{-12}$	»
K_a » glycylglycocolle	$3{,}3 \times 10^{-9}$	—	$1{,}8 \times 10^{-8}$	Euler
K_b » »	$0{,}95 \times 10^{-11}$	$1{,}6 \times 10^{-11}$	2×10^{-11}	»

Les mesurages effectués par M. S. Palitzsch (voir Tableau 1b) ont abouti à un chiffre sensiblement plus élevé pour le K_a de la glycocolle (savoir: environ $1{,}2 \times 10^{-10}$). Il paraît assez probable que cette divergence s'explique par l'«effet de sel», puisque la concentration totale en ions sodium employée par cet auteur est plus forte que dans nos essais.

Comme K_a augmente probablement un peu avec la température (voir le mémoire précité de Lundén), la concordance des valeurs trouvées sera sans doute meilleure pour la glycocolle qu'elle ne l'est dans le Tableau 5, tandis qu'en ce qui concerne la glycylglycocolle notre valeur de K_a est indubitablement moins élevée que celle trouvée par Euler.

En comparant entre elles les différentes valeurs de K_b, il faut tenir compte de ce fait, que la constante de dissociation de l'eau, K_e, entre dans l'expression de cette quantité, et que K_e varie considérablement avec la température (à 18°: $0{,}72 \times 10^{-14}$, à 25°: $1{,}11 \times 10^{-14}$[2]). Réduites à 25°, nos valeurs de K_b, aussi bien de la glycocolle que de la glycylglycocolle, concordent très bien, nous l'avons vu, avec celles constatées antérieurement.

La préparation de glycocolle employée par nous provenait des fabriques de Kahlbaum et contenait la proportion calculée d'azote.

La glycylglycocolle avait été préparée à partir de la glycocolle anhydre (dikétopipérazine), préparée elle-même soit suivant

[1]) Après avoir donné à l'impression le présent exposé, l'auteur a eu connaissance d'un mémoire de Michaëlis (Bioch. Zeitschrift, **49**, 248, 1913), où les constantes de dissociation de la glycocolle se trouvent déterminées comme suit à 17,5°: $K_a = 1{,}2 \times 10^{-10}$ $K_b = 1{,}9 \times 10^{-12}$. Il paraît que ces résultats s'accordent assez bien avec les nôtres.

[2]) Ces mêmes Comptes-rendus, **8**, 1 (1909).

la méthode de Fischer[1]) par M. le Dr Hammarsten, soit au moyen d'un produit fourni par Kahlbaum. Dans l'un et l'autre cas, on a procédé par les méthodes habituelles du Laboratoire de Carlsberg, qui peuvent être résumées ainsi:

On fait dissoudre 4 gr. d'anhydride de glycocolle dans 200 cc. d'une solution saturée de baryte, puis on laisse en repos jusqu'à ce que — au bout de six à huit heures — une prise prélevée sur la solution obtenue et qu'on soumet à la titration au formol permette de constater que la moitié, ni plus ni moins, de l'azote total s'est convertie en azote aminé. Après élimination complète de la baryte au moyen de l'acide sulfurique, on réduit la liqueur par évaporation dans le vide jusqu'au volume le plus petit possible, pour la filtrer ensuite et la faire cristalliser dans le vide de l'exsiccateur. On a obtenu ainsi un rendement de $4^{gr},3$. En vue d'une purification plus parfaite, la glycylglycocolle fut dissoute dans un peu d'eau et précipitée au moyen de l'alcool. La teneur en azote des préparations obtenues, soit 22,10 à 21,18 pour cent, concorde sensiblement avec la proportion calculée: 21,21 pour cent.

B. **Calcul de la concentration en ions hydrogène de mélanges de glycylglycocolle, de glycocolle et d'hydroxyde de sodium.**

La glycylglycocolle se scinde en deux molécules de glycocolle avec fixation d'une molécule d'eau d'après la formule suivante:

$$\begin{array}{l} CH_2 \cdot NH_2 \\ | \\ CO \qquad OH \\ \cdots | \cdots + \cdots \\ NH \qquad H \\ | \\ CH_2 \cdot COOH \end{array} = 2\ NH_2 \cdot CH_2COOH.$$

Connaissant le K_a tant de la glycylglycocolle que de la glycocolle, on est à même de calculer la concentration en ions hydrogène d'un mélange quelconque de ces corps avec l'hydroxyde de sodium.

Supposons qu'une solution de glycylglycocolle dont la concentration primitive serait C subisse l'hydrolyse. Désignons par

[1]) Berichte der deutschen chem. Gesellschaft, **34**, 2868 (1901).

a la concentration que possédera au bout d'un certain temps la glycylglycocolle restante, et par b la concentration de la glycocolle formée. Entre a et b nous trouverons alors la relation suivante:

$$C = a + \frac{b}{2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(I)}.$$

Appelons n la concentration de l'hydroxyde de sodium, h celle des ions hydrogène, k_1 le K_a de la glycylglycocolle, et k_2 le K_a de la glycocolle.

La concentration de la glycylglycocolle dissociée et de celle non-dissociée seront désignées, respectivement, par a_1 et a_2, celles de la glycocolle par b_1 et b_2. La dissociation des deux sels sodiques est supposée complète. Les deux équilibres suivants doivent donc se réaliser:

$$\text{Glycocolle non-diss.} \rightleftharpoons \text{Glycocolle diss.}^{\div} + H^+$$
$$\text{Glycylglycocolle non-diss.} \rightleftharpoons \text{Glycylglycocolle diss.}^{\div} + H^+.$$

Dans ces conditions nous arriverons aux équations suivantes:

$$h \cdot b_1 = k_2 \cdot b_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(II)}$$
$$h \cdot a_1 = k_1 \cdot a_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(III)}$$
$$a_1 + a_2 = a \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(IV)}$$
$$b_1 + b_2 = b \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(V)}$$
$$a_1 + b_1 = n \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(VI)}.$$

Connaissant a, b, n, k_1 et k_2, on peut de ces équations dégager les cinq inconnues a_1, a_2, b_1, b_2 et h.

On trouve h de la manière suivante:

III et IV donneront:

$$h \cdot a_1 = k_1 (a - a_1)$$

et II et V:

$$h \cdot b_1 = k_2 (b - b_1) \quad \text{ou}$$

$$a_1 = \frac{a \cdot k_1}{h + k_1} \quad \text{et}$$

$$b_1 = \frac{b \cdot k_2}{h + k_2}$$

En substituant ces valeurs dans l'équation VI, on aura

$$n = \frac{a \cdot k_1}{h + k_1} + \frac{b \cdot k_2}{h + k_2} \quad \ldots\ldots\ldots\ldots \text{(VII)}.$$

Cette formule est susceptible d'être étendue à un nombre quelconque de composants. Toutefois, elle n'est absolument exacte que dans la supposition que = 1, approximation que, comme il a déjà été mentionné, nous avons faite ici.

En calculant h au moyen de l'équation VII, nous aurons:

$$h = \frac{k_1 (a - n) + k_2 (b - n)}{2n}$$

$$\pm \sqrt{\left[\frac{k_1 (a - n) + k_2 (b - n)}{2n}\right]^2 + \frac{k_1 k_2 (a + b - n)}{n}} \quad \text{(VIII)}.$$

Pour $a = o$ on aura $h = \frac{b - n}{n} k_2$ ..... (IX) et

— $b = o$ — — $h = \frac{a - n}{n} k_1$ ..... (X).

Afin de vérifier l'exactitude de la formule, j'ai préparé des mélanges de glycylglycocolle, glycocolle et hydroxyde de sodium, tels que C était toujours égal à $a + \frac{b}{2}$, correspondant ainsi à une hydrolyse continue, et dans ces mélanges j'ai déterminé la concentration en ions hydrogène suivant la méthode précédemment indiquée. Les concentrations des composants se trouvent consignées dans le Tableau 6, sous a, b et n: les deux colonnes surmontées de l'indication commune $p_H{}^+$, désignant le logarithme, avec le signe négatif sous-entendu, de la concentration en ions hydrogène, contiennent l'une les valeurs constatées et l'autre les chiffres calculés.

Supposant $\alpha = 1$, j'ai employé les valeurs y correspondant de k_1 et k_2 (Tableau 1a et 3).

Dans le Tableau 6 N°s 1—6 on a fait varier a et b, en maintenant constant n; pour les N°s 7—9 c'est l'inverse qui a eu lieu. Dans l'un et l'autre cas, les valeurs trouvées concordent assez bien avec celles qu'avait données le calcul.

TABLEAU 6.

A 18°.

N°	$10^2 \times$ Concentration de			Force électromotrice lue	P_H+	
	Glycylglycocolle a	Glycocolle b	NaOH n	π	Observé	Calculé
1	10	0	4	0,8147	8,213	8,224
2	8	4	4	0,8244	8,395	8,395
3	6	8	4	0,8351	8,579	8,610
4	4	12	4	0,8531	8,903	8,886
5	2	16	4	0,8665	9,134	9,097
6	0	20	4	0,8845	9,445	9,398
7	4	12	1	0,7952	7,878	7,875
8	4	12	2	0,8181	8,272	8,298
9	4	12	4	0,8531	8,903	8,886

Le Tableau 6 permet de tirer cette conclusion importante, que dans une hydrolyse de glycylglycocolle, aux concentrations indiquées ci-dessus, $p_H{}^+$ augmente d'une façon continue de 0,12 environ par 10 pour cent de matière hydrolysée, de sorte qu'à la fin de la réaction $p_H{}^+$ sera environ de 1,2 plus élevé qu'au début.

Du reste, un simple calcul montre qu'il en est sensiblement de même pour un mélange quelconque de glycylglycocolle ayant une concentration initiale en ions hydrogène telle que $p_H{}^+$ se trouve comprise entre 7 et 8.

Les décompositions enzymatiques ont généralement lieu, on le sait, à des températures assez élevées, soit entre 35 et 45°, et, comme nous l'avons vu, k_1 et k_2 varient avec la température; les dosages suivants de l'azote aminé, ainsi que nos déterminations de la concentration en ions hydrogène, ont cependant été effectuées à la température ordinaire du laboratoire.

Dans ce qui va suivre, nous allons d'abord rechercher quelle est l'influence exercée par les variations de la concentration en ions hydrogène sur la vitesse avec laquelle s'opèrent les scissions en question. L'idéal serait d'opérer sur un corps tel que la dite concentration ne subirait pas de modifications au cours de la réaction. Pour y parvenir, il faut se placer dans les deux conditions suivantes: La première est, que les valeurs de h trouvées au moyen des deux équations IX et X doivent être égales, ce qu'on est ordinairement à même d'obtenir en choisissant des concentrations convenables. La seconde condition exige que la valeur ainsi trouvée coïncide avec une concentration en ions hydrogène où l'enzyme considérée est encore active. C'est cependant là une condition que les dipeptides connues ne permettent pas de réaliser, et il est bien douteux que, par voie synthétique, l'on soit à même d'en préparer une qui le permettrait.

En posant $b = 2a$ (p. 273), les équations IX et X donneront

$$\frac{k_1}{k_2} = \frac{2a - n}{a - n} \quad \text{ou} \quad a = \frac{k_1 - k_2}{k_1 - 2k_2} n$$

et, dans les cas où $k_1 > 2k_2$, a et n peuvent toujours être choisis tels qu'ils satisferont aux conditions de cette équation. En substituant cette valeur de a, p. ex. dans X, on aura:

$$h = \frac{k_1 k_2}{k_1 - 2k_2}$$

comme la valeur de h à laquelle la solution d'une dipeptide quelconque ne changerait pas de concentration en ions hydrogène, lorsque la dipeptide subit la scission. Mais cette concentration n'est généralement point située dans les régions où l'enzyme est douée d'un pouvoir actif. Si, p. ex., on fait entrer dans cette expression les valeurs de k_1 et k_2 de la glycylglycocolle consignées dans le Tableau 5, on aura:

$$h = 1{,}09 \cdot 10^{-10},$$

valeur qui est fort éloignée de la région où l'enzyme considérée exerce quelque action.

C. **Préparation de l'enzyme.**

A moins d'indication contraire, nous nous sommes constamment servi de jus dialysé de levure, préparé suivant le procédé indiqué par Koelker[1]) et qui peut être résumé comme suit:

500 gr. de levure de bière, soigneusement lavée, puis pressée, sont triturés dans un mortier avec 30 gr. de carbonate de calcium et 30 cc. de chloroforme. On fit subir à ce mélange une autolyse durant trois jours à la température du laboratoire, puis ensuite on le pressa au travers de canevas, pour le soumettre enfin à la filtration. — D'après la prescription de l'auteur cité, il conviendrait alors d'abandonner le jus ainsi obtenu pendant deux jours à la température de 38°; cependant nous avons trouvé qu'il perd ainsi beaucoup de son efficacité, et qu'il vaut mieux ne pas le soumettre à une température aussi élevée.

Plus tard nous fîmes subir à la liqueur pendant six jours, une dialyse sous pression réduite (suivant des procédés imaginés au Laboratoire de Carlsberg et qui n'ont pas encore été publiés), en employant le toluène comme agent de conservation. On vit tomber des matières albuminoïdes. Par filtration on obtint un liquide limpide d'une couleur jaune clair, qui vraisemblablement contenait la totalité de l'enzyme, attendu que l'eau de dialyse ne montrait pas de pouvoir protéolytique. La préparation fut conservée en contact de toluène dans la glacière.

Pour les opérations que nous allons relater, nous avons fait principalement usage de deux préparations obtenues de la manière que nous venons de décrire. Pour abréger, elles seront désignées dans les tableaux, respectivement, par Enzyme a et Enzyme b.

[1]) Zeitschrift für physiologische Chemie, **67**, 297 (1910).

La première, qui avait été préparée au moyen de levure provenant de la brasserie de Carlsberg, avait subi l'action de la température prescrite par Koelker (38°), et se trouva être relativement faible. Sa teneur totale en azote se montait à $0^{mgr},9$ par centimètre cube, dont seulement une proportion extrêmement minime put être titrée au formol. Une vingtaine de jours après la dialyse, le pouvoir de scission possédé par cette préparation se trouva avoir diminué de moitié environ.

L'«enzyme b», pour laquelle on avait eu recours à de la levure fournie par la Brasserie de St: Erik à Stockholm, et qui n'avait pas été traitée à 38°, fit preuve d'une force d'action de beaucoup plus marquée. La teneur totale en azote par centimètre cube était de $0^{mgr},8$; elle ne renfermait que des traces d'azote aminé. Après quarante jours, son pouvoir actif s'était abaissé à un cinquième de ce qu'il était au début, et il s'était formé $0^{mgr},4$ d'azote aminé par centimètre cube.

Ces préparations ne sauraient, à la vérité, être considérées comme des solutions d'enzyme parfaitement pure; cependant, vu que les protéines qu'elles peuvent renfermer ne paraissent pas subir de décomposition ultérieure, j'estime pouvoir les regarder comme étant d'une pureté satisfaisante. La concentration en ions hydrogène optimale est située à $p_H{}^+ = 7,8$ (voir ci-dessous), et l'action cesse presque tout à fait vers le point neutre. Cette enzyme n'est donc pas identique à l'«endotryptase», enzyme que Hahn et Geret[1]) ont signalée les premiers et qui n'exerce son action qu'en milieu acide. Elle diffère également des enzymes qui produisent l'autolyse de la levure, réaction étudiée antérieurement par l'auteur du présent exposé[2]) et qu'il a reconnue n'avoir lieu qu'en solution acide.

A tout prendre, la plus proche parente de notre enzyme paraît être l'érepsine, signalée par Cohnheim dans le suc entérique. En conséquence, d'accord avec la terminologie généralement reçue, il conviendra de la nommer l'éreptase de levure.

Pour ce qui concerne la température optimale, nous n'avons pas jusqu'ici fait de recherches. En ce qui regarde d'autres enzymes[3]),

[1]) Buchner und Hahn: Die Zymasegärung (Munich 1903).
[2]) Zeitschrift für physiol. Chemie, **89**, 406 (1914).
[3]) A. Compton: Roy. Soc. Proc. B. Vol. **88**, 408 (1915).

on a constaté que la concentration en ions hydrogène optimale varie avec la température. C'est pourquoi tous nos essais ont été effectués à 38°, avec une déviation de $\pm$ 0°. 1.

D. **Configuration des courbes de scission à des concentration différentes et variables en ions hydrogène.**

Les expériences ont porté sur des solutions contenant 10 cc. de glycylglycocolle normale aux $^2/_5$ mélangée avec de l'hydroxyde de sodium en proportion telle, que la concentration voulue en ions hydrogène en résultait dans chaque cas particulier. On y ajoutait 3 cc. de solution enzymatique (voir Tableau 7), et la liqueur était étendue jusqu'à concurrence de 100 cc. avec de l'eau distillée additionnée de chloroforme. On opérait la décomposition dans des fioles Erlenmeyer d'une capacité de 200 cc., qu'on plaçait dans une étuve où la susdite température de 38° était maintenue. On n'ajoutait cependant l'enzyme au mélange indiqué que lorsque celui-ci avait atteint la température de l'étuve, et c'est à partir de ce moment que nous comptons la durée de chaque essai.

Aux heures indiquées dans les tableaux, on prélevait des prises de 20 cc. chacune, dont l'azote aminé fut dosé par titration au formol d'après Sörensen[1]). La concentration initiale en ions hydrogène fut calculée ou bien déterminée colorimétriquement à l'aide de mélanges de phosphates[2]) comme liqueurs de comparaison.

Sous la rubrique «%N», les tableaux qui suivent indiquent le pourcentage d'azote aminé formé, sous «K» les résultats numériques qu'a donnés le calcul de la constante de réaction monomoléculaire:

$$K = \frac{1}{t} \ln \frac{a}{a-x}.$$

En portant le pourcentage d'azote libéré en ordonnée, et le nombre d'heures en abscisse, on arrive à la figure 1.

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[1]) Voir Jessen-Hansen: Die Formoltitration. Abderhaldens Handbuch der bioch. Arbeitsmethoden, **6**, 270.

[2]) Ces mêmes Comptes-rendus, **8**, 1 (1909).

TABLEAU 7.

10 cc. glycylglycocolle 0,4-norm. + NaOH 0,2-norm. + 3 cc. enzyme (a) + eau = 100 cc. $t = 38°$

N°	I		II		III		IV		V	
cc. NaOH 0,2-n.	1		2		3		8		10	
p_H+ initial	7,14		7,47		7,66		8,24		8,42	
Au bout de — heures	%N	K	%N	K	%N	K	%N	K	%N	K
0	0	--	0	—	0	—	0	—	0	—
3	3	0,009	6	0,021	9	0,030	12	0,042	12	0,042
7	8	0,012	15	0,025	18	0,028	27	0,046	21	0,035
22	26	0,014	43	0,025	38	0,021	44	0,028	32	0,018
31	32	0,014	54	0,025	50	0,023	47	0,021	33	0,012

L'inspection des courbes fait voir immédiatement que la marche de la réaction est très différente selon la concentration initiale en ions hydrogène: Les courbes I et II sont presque rectilignes, alors que III, IV et V montrent des incurvations de plus en plus prononcées, et il apparaît que la vitesse diminue au fur et à mesure que la réaction avance.

Il ressort de ces courbes que l'action enzymatique est essentiellement sous la dépendance de la concentration en ions hydrogène. La concentration optimale se trouve bien nettement aux environs de $p_H{}^+ = 8$; pourtant elle ne peut pas être déterminée avec exactitude de cette façon attendu que, comme nous l'avons vu, $p_H{}^+$ varie de 0,12 environ pour 10 pour cent de matière scindée.

Il va de soi que nos courbes, qui au surplus se coupent les unes les autres, ne sauraient s'exprimer par une formule mathématique commune. Dans les courbes I et II, la constante de réaction augmente, tandis que dans les trois autres elle diminue, fait qui eût été encore plus prononcé, si la concentration de l'enzyme avait été plus grande et la scission s'était produite plus rapidement.

Ces mêmes courbes nous donnent aussi l'explication des résultats contradictoires auxquels sont arrivés Abderhalden et Michaëlis ainsi que Euler[1]) dans leurs recherches sur la scis-

[1]) Aux endroits cités.

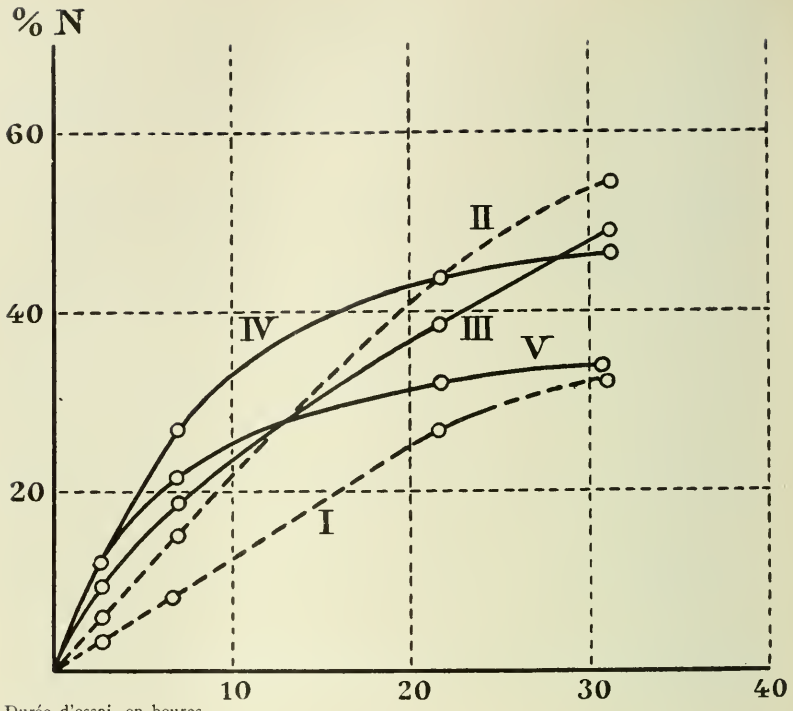

Fig. 1.

sion enzymatique de la glycylglycocolle. Dans le mémoire de Abderhalden et Michaëlis on ne trouve aucune indication relative à l'addition d'un alcali, et il paraît donc légitime de conclure que la concentration en ions hydrogène était supérieure à celle qui convient le mieux à l'enzyme expérimentée. Plus la réaction progresse, plus le liquide devient alcalin, et plus grande, par conséquent, sera la partie de l'enzyme qui devient active. Il est évident que dans ces conditions la constante de réaction doit s'accroître.

Quant à Euler, il est vrai qu'il a ajouté un alcali, mais dès le commencement de la réaction il semble avoir dépassé l'optimum de la concentration en ions hydrogène. Calculés d'après la formule X (p. 274), ses chiffres indiquent que dans ses essais $p_H{}^+$ a été situé entre 8 et 8,5. Les essais sont donc comparables à IV et V.

Il appert de ces considérations que dans les recherches sur les enzymes protéolytiques il n'est pas moins important de maintenir constante la concentration en ions hydrogène que la tem-

pérature à laquelle on opère, et l'on voit en outre, qu'il est nécessaire de maintenir cette concentration dans des limites où l'enzyme ne soit pas sujette à une forte autodécomposition.

E. **Concentration en ions hydrogène optimale pour l'éreptase de levure.**

Dans le but d'établir quelle était la concentration en ions hydrogène optimale pour la scission enzymatique en question, je procédai aux essais dont les résultats sont réunis dans le Taubleau 8. Afin de maintenir constante ladite concentration pendant la durée de la réaction, j'eus soin d'ajouter un «tampon» approprié, à savoir les mélanges de phosphates $^{M}/_{5}$ primaire et secondaire d'après Sörensen. A 10 cc. de solution 0,4-n. de glycylglycocolle + z cc. de lessive de soude 0,2 n. + 5 cc. de solution d'enzyme (b) j'ajoutai 20 cc. de phosphates (s cc. de phosphate secondaire + p cc. de primaire), en complétant le mélange avec de l'eau distillée jusqu'à 100 cc. (Les valeurs de z, s et p, qui dépendent de la concentration voulue en ions hydrogène, se trouvent indiquées dans les tableaux). Des essais préliminaires avaient montré que cette concentration en phosphates suffisait pour maintenir $p_H{}^+$ constant avec des déviations de $\pm$ 0,1, au moins en ce qui concerne I à IV, et, de plus, elle fut reconnue n'entraver en rien l'action de l'enzyme.

La scission fut réalisée, comme auparavant, à 38°, avec addition de chloroforme. Chaque échantillon était, en général, de 15 cc.; on élimina l'acide phosphorique[1]) en diluant jusqu'à 50 cc., puis on fit le titrage au formol sur 40 cc., correspondant à 12 cc. de l'échantillon. Comme $0^{cc},05$ de lessive de soude normale au $^{n}/_{5}$ correspond ici à 2 pour cent d'azote aminé, les pourcentages sont donnés dans les tableaux en nombres entiers.

Au Tableau 8 se trouve calculée, sous «K», la constante de réaction monomoléculaire. On voit que dans les séries I à III cette quantité va en augmentant, tandis que dans IV et V elle décroît. Dans ce dernier cas la concentration optimale en ions hydrogène a été dépassée, ce qui a probablement pour effet que l'enzyme subit plus rapidement la destruction. Nous reviendrons plus tard sur cette question.

---

[1]) Voir Jessen-Hansen: Die Formoltitration, à l'endroit cité.

TABLEAU 8.

10 cc. de glycylglycocolle (0.4 n) + 5 cc. d'enzyme (b) + z cc. de Na OH $N/_5$ + 20 cc. de phosphate + eau = 100 cc. t = 38°.

N°	I		II		III		IV		V	
z	0		2		4		6		8	
s + p	10 + 10		16,4 + 3,6		18,4 + 1,6		19,0 + 1,0		19,5 + 0,5	
p_H +	6,80		7,47		7,82		8,00		8,24	
Au bout de — heures	%N	K	%N	K	%N	K	%N	K	%N	K
0	0	—	0	—	0	—	0	—	0	—
3/4	—	—	28	0,45	41	0,70	38	0,64	34	0,55
1 1/2	18	0,13	50	0,46	68	0,76	59	0,52	52	0,54
3	36	0,14	82	0,55	91	0,80	82	0,55	75	0,46
4 1/2	53	0,17	92	0,56	(100)	—	91	0,53	79	0,35
6	68	0,18	(100)	—	—	—	95	0,50	82	0,31

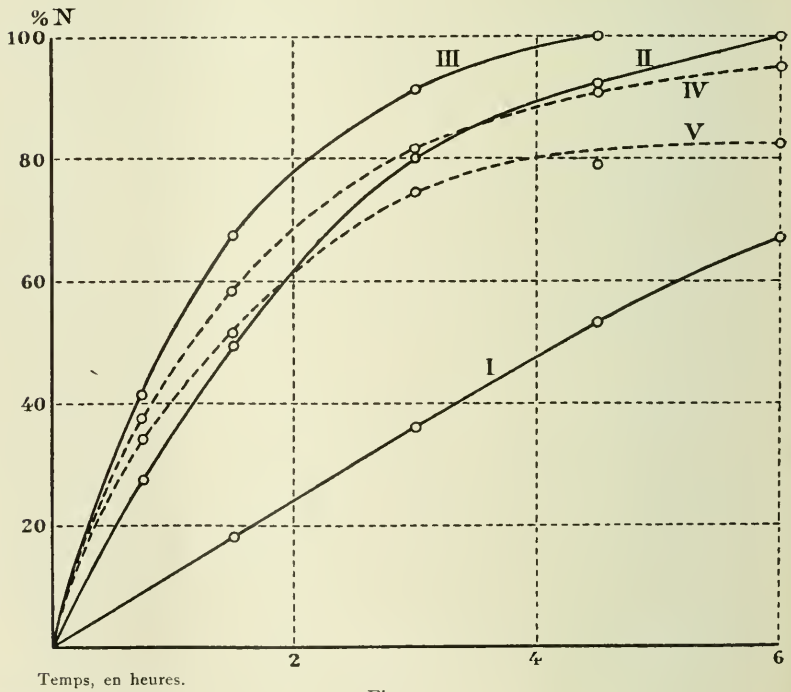

Fig. 2.

Si, à l'exemple de ce que Sörensen[1]) a fait pour d'autres réactions enzymatiques, nous portons en ordonnée le temps demandé pour scinder une certaine partie de la glycylglycocolle, et en abscisse l'exposant des ions hydrogène (p_H^+) régnant dans l'essai, nous obtiendrons les courbes suivantes (Tableau 9 et figure 3), respectivement pour 50 et 75 pour cent.

TABLEAU 9.

p_H^+	50 % d'azote ont été libérés en — heures	75 % d'azote ont été libérés en — heures
6,8	4,3	env. 8
7,47	1,5	2,6
7,82	0,95	1,8
8,00	1,2	2,1
8,24	1,4	2,6

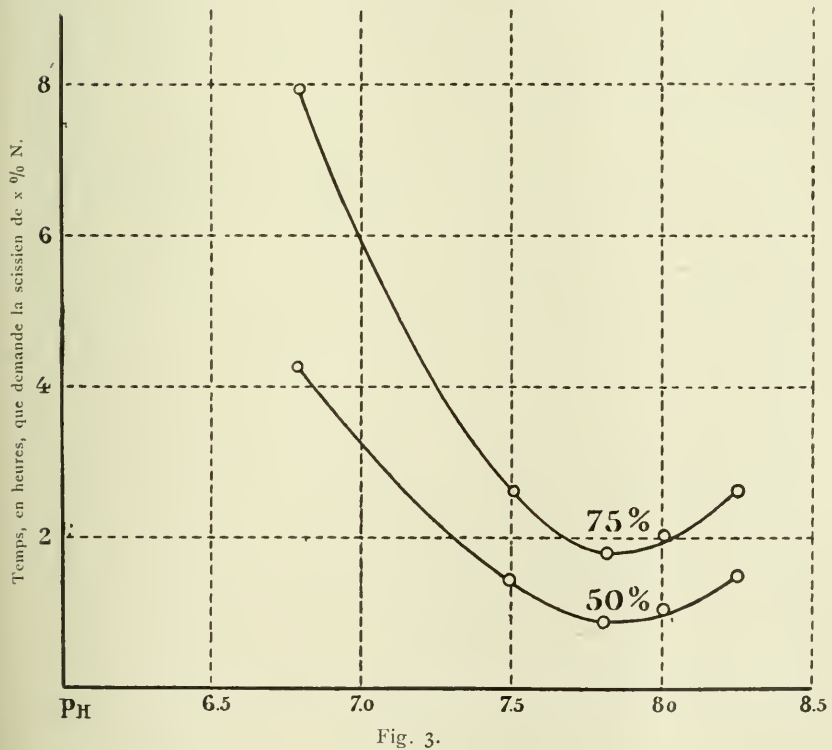

Fig. 3.

[1]) Ces mêmes Comptes-rendus, **8**, 1 (1909).

Le minimum de la courbe correspond donc ici à la concentration optimale en ions hydrogène, et celle-ci se trouve bien nettement à $p_H{}^+ = 7{,}8$, résultat que Sörensen et Walther ont également obtenu dans un travail qui n'a pas encore été publié.

On arrive à une courbe tout à fait analogue si, comme Rona et Arnheim[1]) l'ont fait pour l'érepsine, nous choisissons pour ordonnée φ/Φ, où Φ représente la quantité arbitrairement choisie d'enzyme à l'optimum de $p_H{}^+$, et φ celle agissant à une certaine autre valeur de $p_H{}^+$. Le rapport ne peut pas être déterminé directement, contrairement à T/t, c'est-à-dire la raison inverse entre les temps qu'exige la production d'une même quantité des produits de scission à l'optimum de $p_H{}^+$ et à une autre valeur de $p_H{}^+$. On peut conclure par analogie, paraît-il, que la courbe dont il s'agit est bien la courbe de dissociation d'un acide dont la constante de dissociation est $10^{-7{,}2}$, ou à peu près. Vu les fautes de lecture assez graves, vu aussi le petit nombre des points observés, cette courbe ne peut être regardée que comme approximativement exacte; c'est pourquoi l'auteur a cru pouvoir se dispenser de la tracer.

F. **Allure des courbes de scission à la concentration en ions hydrogène optimale et constante.**

Dans le chapitre précédent nous avons vu que, si la concentration ionique est constante, les courbes de scission suivent approximativement la formule monomoléculaire, tant que le point optimum n'est pas dépassé vers le côté alcalin. Afin de vérifier l'exactitude de ce résultat, nous avons procédé aux essais que nous allons relater et dont les résultats se trouvent consignés dans les Tableaux 10 et 11. La liqueur expérimentée contenait par 100 cc., 20 cc. de mélange de phosphates M/5 (18cc,4 de secondaire et 1cc,6 de primaire), de sorte que $p_H{}^+$ était constamment de 7,8. Pour chaque titrage au formol je pris un échantillon de 25 cc., ce qui a réduit la faute de lecture à 1 pour cent, au plus.

J'ai choisi une concentration de l'enzyme telle que la réaction marche avec une lenteur suffisante pour qu'une erreur de quelques minutes dans les intervalles entre les prises d'essais ne puisse pas avoir d'influence appréciable. Ainsi qu'on peut le voir

[1]) Biochemische Zeitschrift, **57**, 84 (1913).

TABLEAU 10.

10 cc. glycylglycoc. 0,4-n. + 4 cc. NaOH 0,2-n. + 1 cc. enzyme (b) + 20 cc. phosph. + eau = 100 cc.

$p_H+ = 7,8$ $t = 38°$

Heures	% N aminé x	K
0	0	—
1	13,8	0,157
2	25,9	0,150
3	36,2	0,150
4	44,9	0,150
5	55,2	0,157
8	69,0	0,145
10	75,9	0,143
12	82,8	0,147
24	96,6	(0,138)

TABLEAU 11.

10 cc. glycylglycoc. 0,4-n. + 4 cc. NaOH 0,2-n. + 10 cc. enzyme (a) + 20 cc. phosph. + eau = 100 cc

$p_H+ = 7,8$ $t = 38°$

Heures	% N aminé x	K
0	0	—
3	28,6	0,113
6	50,1	0,115
9	62,0	0,108
21	90,6	0,113
30	93,0	(0,090)

par les tableaux, les valeurs de K sont on ne peut plus constantes, eu égard à l'erreur de lecture. Dans le Tableau 10, K accuse pourtant une légère tendance à diminuer, fait sur lequel nous aurons à revenir plus loin.

Le Tableau 12 et la figure 4 donnent les résultats de cinq essais analogues, dans lesquels la concentration de l'enzyme variait cependant de 0,5 à 5. Les essais II à V s'accordent avec la formule de réaction monomoléculaire; seule I montre une légère diminution de la constante.

Vu que ces courbes sont logarithmiques, elles doivent se conformer à la loi bien connue dite de $q - t$[1]), si q représente la concentration de l'enzyme, et t le temps exigé dans chaque cas particulier par la scission d'une même quantité de corps à scinder. Il ressort du Tableau 12, ce qui revient au même, que le produit de 1/K et de la concentration de l'enzyme est, dans tous les cas considérés, constant, ou peu s'en faut.

Dans le Tableau 12, I, nous voyons la valeur de K diminuer avec le temps. Le Tableau 13 donne le résumé d'un essai analogue, mais plus exact, où la concentration de l'enzyme est très peu élevée par rapport au corps à scinder.

[1]) Arrhenius: Quantitative Laws of Biological Chemistry, p. 44 (London 1915).

TABLEAU 12.

10 cc. 0,4-n glycylglycocolle + 2 cc. 0,2-n. NaOH + x cc. enzyme (b) + 20 cc. phosphates + eau = 100 cc.

$p_H+ = 7,8$ $t = 38^0$

Heures	I x = 0,5		II x = 1		III x = 2		IV x = 3		V x = 5	
	% N	K	% N	K	% N	K	% N	K	% N	K
0	—	—	—	—	—	—	—	—	—	—
1/2	—	—	—	—	—	—	—	—	32	0,79
1	—	—	14	0,15	23	0,35	32	0,40	55	0,80
2	14	0,074	—	—	41	0,27	59	0,45	77	0,74
3	—	—	34	0,14	59	0,30	77	0,49	91	0,80
4	—	—	—	—	—	—	—	—	(100)	—
5	30	0,071	50	0,14	82	0,35	92	0,51	—	—
9	—	—	73	0,15	(100)	—	(100)	—	—	—
12	50	0,058	—	—	—	—	—	—	—	—

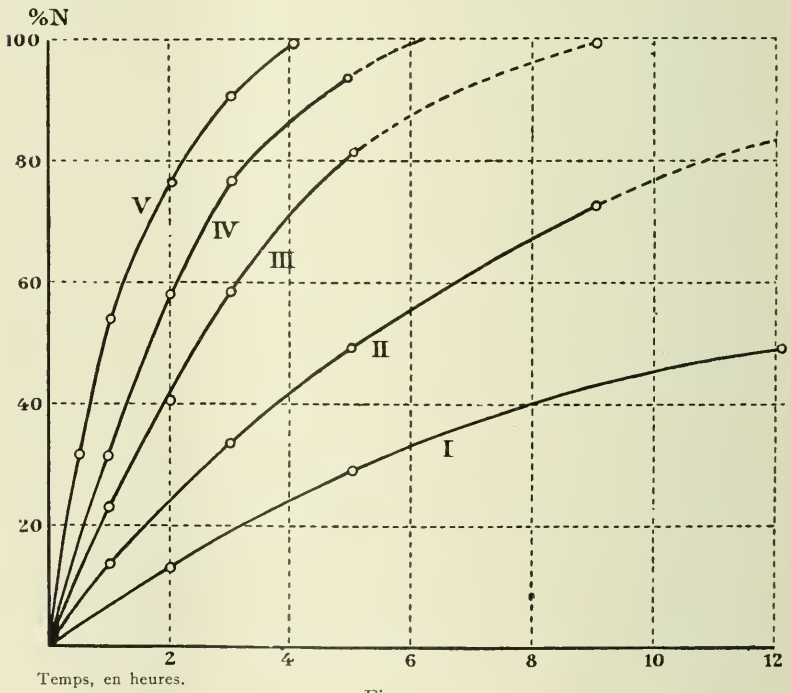

Fig. 4.

TABLEAU 13.

0,5 cc. d'enzyme (b) dans 100 cc.
$p_H+ = 7,8$ $t = 38^0$

Heures	% d'azote aminé x	K
0	0	—
2	14	0,074
5	29,5	0,071
12	50,0	0,058
24	59,1	0,037
48	68,2	0,023
72	68,2	0,016)

Il apparaît que K décroît au fur et à mesure que la réaction progresse. Lorsque 68 pour cent de l'azote ont subi la scission, l'action cesse tout à fait, c'est-à-dire que l'enzyme s'est épuisée entièrement.

En résumé, il paraît résulter avec certitude de ces essais que, tant que la concentration en ions hydrogène demeure constante et que le point optimal n'aura pas été dépassé, la scission de la glycylglycocolle au moyen de l'éreptase de levure est une réaction monomoléculaire, pourvu que la concentration de l'enzyme par rapport au corps à scinder soit suffisamment forte pour que l'autodécomposition de l'enzyme puisse être négligée.

G. **Autodécomposition de l'enzyme en milieu alcalin.**

En concentration faible, l'autodécomposition de l'enzyme exerce toujours une influence sensible sur l'allure des courbes de scission. Afin de nous faire une idée de l'étendue de cette autodécomposition, nous avons entrepris les essais résumés dans la figure 5.

Les courbes reproduites ici représentent des scissions analogues aux précédentes. La courbe I montre comment les choses se passent lorsque p_H+ reste constamment égal à 7,8. Dans les essais représentés par II on n'avait pas ajouté de «tampon», la valeur de p_H+ étant de 8,3. Lorsque environ un quart de la glycylglycocolle eut été scindé et que, par conséquent p_H+, eut

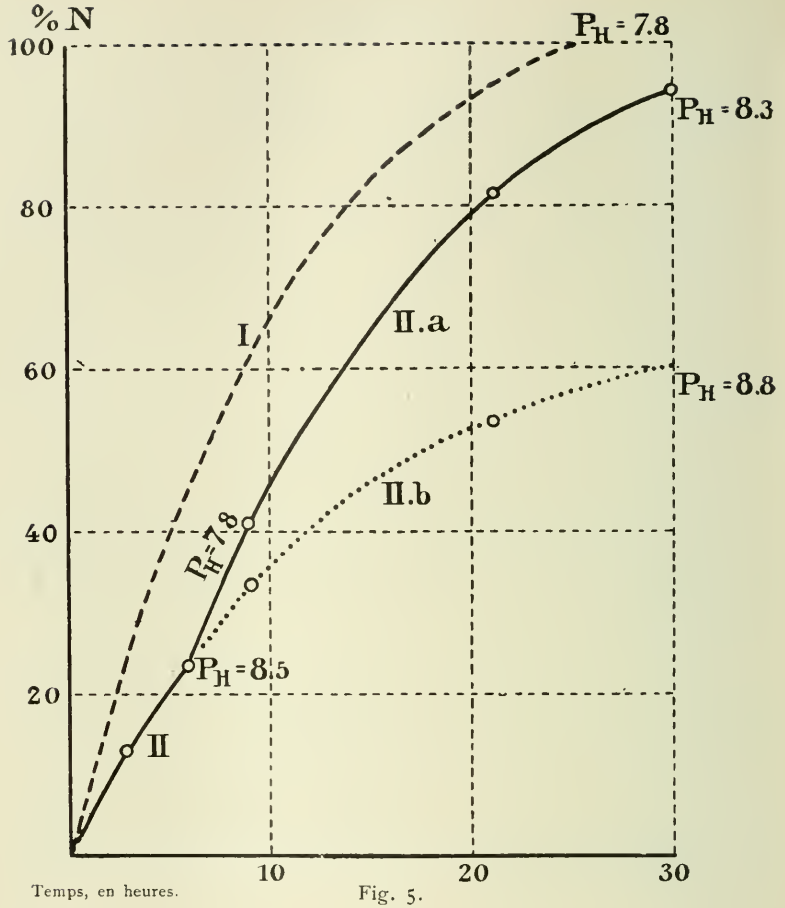

Fig. 5.

atteint le voisinage de 8,5, on partagea le liquide en deux parties, et à l'une d'elles on ajouta avec précaution de l'acide chlorhydrique en proportion telle que $p_H{}^+$ fût réduit à 7,7. C'est la marche qu'a prise la réaction dans cette portion qui se trouve indiquée par IIa, tandis que IIb montre celle observée dans l'autre partie du liquide. On voit ici que, à n'en pas douter, il s'agit bien d'un phénomène de dissociation, attendu que l'action enzymatique éprouve un affaiblissement lorsque la valeur de $p_H{}^+$ dépasse l'optimum. Mais on voit aussi que le milieu trop alcalin a déterminé une destruction partielle de l'enzyme.

En vue de rechercher jusqu'à quel point un traitement préliminaire en milieu alcalin à 38° pourrait influer sur le pouvoir actif de l'enzyme, j'ai effectué l'essai suivant:

A 25 cc. de la solution d'enzyme (a) j'ajoutai 92 cc. de phosphate secondaire et 8 cc. de phosphate primaire ($p_H{}^+ = 7{,}8$), puis, après dilution jusqu'à 200 cc., je plaçai de mélange dans l'étuve à 38°. Aux heures indiquées dans le Tableau 14, j'en enlevai 40 cc., que j'additionnai de 5 cc. glycylglycocolle normale aux $^2/_5$ + 2 cc. de lessive de soude normale au $^1/_5$ + 3 cc. d'eau. Dans ces échantillons je déterminai alors le pouvoir de scission, par titration au formol d'après le procédé exposé plus haut. Dans un essai parallèle, on opéra une scission à l'aide d'une enzyme de même concentration et qui n'avait été soumise à aucun traitement préalable (Cet essai est désigné par I dans le Tableau 14).

$p_H{}^+ = 7{,}8$ TABLEAU 14. $t = 38°$

Heures	% d'azote scindé d'une enzyme traitée préalablement en — heures				
	0		3	6	9
	I		II	III	IV
	% N	K			
0	0	—	0	0	0
3	29	0,11	16	10	7
6	50	0,12	29	—	—
9	62	0,11	—	—	—
12	—	—	—	—	33
15	80	0,11	—	43	—
18	—	—	62	—	—
21	91	0,11	—	—	—

Il appert par la figure 6 et le Tableau 14 que le traitement préliminaire tend à diminuer fortement le pouvoir protéolytique, fait que l'on ne peut évidemment s'expliquer qu'en admettant que l'enzyme subit la destruction dans le milieu alcalin. Quant aux quantités de substance détruites au bout d'un même nombre d'heures, elles sont alors proportionnelles à la partie de l'enzyme qui a échappé à la destruction. C'est ainsi qu'après 3—6—9 heures de traitement préliminaire

$$\frac{16 \cdot 100}{29}, \quad \frac{10 \cdot 100}{29} \text{ et } \frac{7 \cdot 100}{29} \text{ pour cent}$$

de l'enzyme se trouvent rester inaltérés. En rassemblant ces valeurs dans un tableau et calculant $K = \frac{1}{t} \ln \frac{a}{a - x}$, où t si-

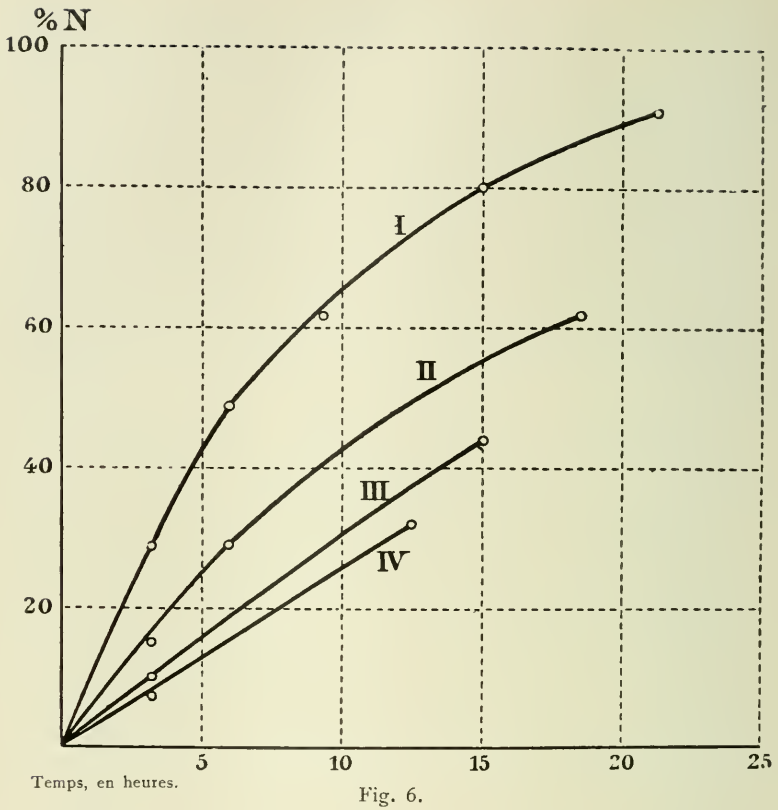

Fig. 6.

gnifie le temps qu'a duré le traitement préalable, et x le pourcentage d'enzyme détruite, on arrive à une valeur relativement constante de K (voir Tableau 15).

TABLEAU 15.

Heures	% d'enzyme x	K
0	0	—
3	45	0,20
6	65	0,18
9	76	0,16

Ainsi donc, il paraît que la destruction de l'enzyme s'opère d'une façon monomoléculaire et avec une vitesse relativement assez grande.

Cependant, une inspection des valeurs consignées au Tableau 14, I de la constante de réaction, trouvées pour la scission produite par une enzyme non traitée au préalable, fait voir que cette quantité ne subit aucune variation, ce qui implique naturellement que la vitesse de destruction est fort minime par rapport à celle de scission.

Ces résultats nous forcent à conclure que le corps à scinder exerce une influence protectrice sur l'enzyme, conclusion qui s'accorde parfaitement avec celle que Vernon[1]) a tirée de ses recherches sur la trypsine et qui consiste à admettre que la stabilité de cette enzyme est augmentée par la présence de matières protéiques et d'acides aminés.

Les faits ainsi constatés font comprendre comment il se fait que la vitesse de réaction pour une même concentration enzymatique augmente avec la concentration du corps à scinder. Dans le Tableau 16 se trouve résumée une expérience effectuée en vue d'élucider cette question. Seulement, au lieu d'indiquer le pour cent d'azote libéré, on a calculé celui-ci en milligrammes par 12 cc. de liquide.

TABLEAU 16.

0,4-n. glycylglycocolle + 0,2-n. Na OH + 20 cc. phosphates + 10 cc. enzyme (a) + eau = 100 cc.

a) 5 cc. glycylglycocolle + 2 cc. Na OH
b) 10 » » + 4 » »
c) 20 » » + 8 » »

$p_H+ = 7,8$ $t = 38^0$

Heures	Mgr. d'azote libéré		
	a	b	c
0	0	0	0
2	0,8	0,8	0,8
7	2,1	2,8	3,1
12	2,8	4,2	5,6
24	2,8	5,3	7,3

A vrai dire, ces essais ne sont que l'inverse de ceux résumés dans le Tableau 12. Dans l'un et l'autre cas, les résultats

[1]) Journal of Physiology, **30**, 31 (1904).

paraissent démontrer que, en réalisant des scissions de la glycylglycocolle par des enzymes protéolytiques, il convient d'employer et l'enzyme et la glycylglycocolle en concentrations telles qu'on n'ait pas besoin de tenir compte de la destruction de l'enzyme. Mais de faire entrer dans l'équation de réaction le coefficient de destruction de l'enzyme, ainsi que Tamman[1]) l'a fait pour de faibles concentrations d'autres enzymes, c'est ce qui est impossible ici, attendu que, comme il résulte des expériences ci-dessus, la destruction de l'enzyme est sous la dépendance de la concentration du corps à scinder.

H. **L'influence qu'exercent certains ions sur la scission.**

Euler[2]) a établi que l'addition de glycocolle n'a pas d'influence sur la scission de la glycylglycocolle par l'érepsine. D'autre part, il ressort du Tableau 17 que dans ce cas aussi ladite addition n'influe pas sur la vitesse de réaction.

TABLEAU 17.

a) 10 cc. 0,4-n. glycylglycocolle + 4 cc. 0,2-n. NaOH + 20 cc. phosphates + 10 cc. enzyme (a) + eau = 100 cc.
b) De même + 10 cc. 0,4-n. glycocolle = 100 cc.

$p_H+ = 7{,}8$ $t = 38^0$

Heures	% N	
	a	b
0	0	0
2	14	16
7	48	48
12	68	71
24	90	88

[1]) Euler-Pope: General Chemistry of Enzymes (1912), p. 233. La vitesse de scission pouvant être exprimée par

$$\frac{dx}{dt} = k_1 (a - x)$$

et la vitesse de destruction de l'enzyme par

$$\frac{dy}{dt} = k_2 (b - y),$$

on arrive, en combinant ces deux formules, puis en faisant l'intégration, à la formule de réaction suivante:

$$K = \frac{e^{k_2 t}}{e - 1} \ln \frac{a}{a - x}.$$

Cependant, dans notre cas, k_2 n'est évidemment pas une constante, mais dépend de la concentration de la glycylglycocolle, et il paraît donc que cette formule ne peut pas servir ici.

[2]) A l'endroit cité.

Le Tableau 18 se rapporte à un certain nombre d'essais où l'on ajoutait des sels ayant les ions inorganiques les plus communs, sels dont la concentration se trouve indiquée dans le Tableau pour chaque liqueur expérimentée. De même que précédemment, on s'est servi de phosphates comme tampon.

TABLEAU 18.

10 cc. 0,4-n. glycylglycocolle + 4 cc. 0,2-n. NaOH + 20 cc. phosphate + 5 cc. enzyme (b) + 10 cc. sol. de sel + eau = 100 cc.

$p_H+ = 7{,}8$ $t = 38°$

Heures	Sans addition de sel	KCl 0,2-n.	NaCl 0,2-n.	KBr 0,2-n.	NaJ 0,2-n.	KJ 0,2-n.	KJ 0,02-n.	NaF 0,2-n.	Na_2SO_4 0,2-n.	$NaNO_3$ 0,2-n.	$KClO_3$ 0,1-n.	KCN 0,1-n.[1])
0	—	—	—	—	—	—	—	—	—	—	—	—
1	55	55	55	55	27	27	36	55	55	55	36	0
2	77	77	77	77	32	32	55	77	77	73	64	0

Parmi les ions, l'anion $CN\div$ exerce l'influence la plus défavorable; les anions $ClO_3\div$ et $J\div$ ont une action sensiblement plus faible, qui devient nulle dans le cas des anions $Cl\div$, $Br\div$, $F\div$, $SO_4\div$, $NO_3\div$, et il en est manifestement de même — du moins pour les concentrations choisies — en ce qui concerné les anions des phosphates employés. La façon dont se comporte ici le fluorure de sodium est assez singulière, attendu que dans d'autres cas ce sel s'est montré bien nettement comme un poison pour les enzymes.

Quant aux cations, mes essais, en raison de l'alcalinité de la liqueur, n'ont porté que sur le potassium et le sodium, avec ce résultat qu'ils paraissent inactifs.

[1]) Comme KCN subit une forte hydrolyse, la valeur de p_H+ était probablement, malgré la présence des phosphates, supérieure à 7, 8; cependant il ne peut guère y avoir de doute que l'ion $CN\div$ n'exerce une action destructive sur l'enzyme. Depuis, on a effectué des essais avec des concentrations moins fortes en KCN, et l'on est arrivé à ce résultat que le pouvoir protéolytique subit un affaiblissement et n'est pas tout à fait annihilé.

Pour ce qui est de l'action ralentissante des ions iode, elle ne s'exerce probablement pas de façon directe, mais plutôt par l'intermédiare d'une sorte d'oxydation donnant lieu à une formation d'iode libre; car une addition d'hyposulfite de sodium à la liqueur expérimentée, qui contenait KJ ou NaJ, n'a pas fait constater une diminution de l'action enzymatique. Du reste, je dois renvoyer le lecteur à un mémoire qui paraîtra prochainement dans les Meddelanden från K. Vet. Akad. Nobelinstitut för fysikalisk kemi.

En cela, notre éreptase de levure semble se distinguer de celle tirée du suc entérique, enzyme dont, d'après Kobzarenko[1]), l'action est entravée par les sels de sodium, mais non par ceux de potassium.

L'auteur a opéré quelques dédoublements de glycylglycocolle par de l'éreptase tirée du suc entérique du cochon, par lesquels il a constaté que l'addition des phosphates fait entraver très considérablement l'action de cette éreptase, tandis que l'éreptase de levure, ainsi qu'on l'a vu dans ce qui précède, n'est pas sujette à une telle influence. Ce fait fera l'objet d'une recherche future.

Résumé.

Le dédoublement enzymatique de la glycylglycocolle au sein d'une solution alcaline amène une diminution de la concentration des ions hydrogène (c'est-à-dire que $p_H{}^+$ s'accroît), diminution qui est d'autant plus grande que la réaction est plus avancée. Cela tient à ce que la glycylglycocolle, en tant qu'acide, a une constante de dissociation (K_a) plus grande que celle de la glycocolle. Nous avons trouvé pour K_a et K_b de ces deux corps les valeurs suivantes:

	K_a	K_b
Glycylglycocolle	$3{,}3 \cdot 10^{-9}$	$0{,}95 \cdot 10^{-11}$
Glycocolle	$1{,}05 \cdot 10^{-10}$	$1{,}7 \cdot 10^{-12}$

Puisque l'enzyme considérée, l'éreptase de levure, peut être regardée comme un électrolyte amphotère qui, en tant qu'acide, possède une constante de dissociation voisine de $10^{-7{,}2}$, son activité est influencée notablement par les variations subies par la concentration en ions hydrogène du milieu. Pour cette raison il est tout aussi important, lors du dédoublement, de maintenir constante cette concentration, que de ne pas laisser varier la température. C'est ce qu'on a obtenu ici en ajoutant aux liquides expérimentés des mélanges des phosphates primaire et secondaire.

A une concentration en ions hydrogène constante et plus grande que celle de l'optimum, l'enzyme agit, pratiquement parlant, comme un catalyseur purement chimique, attendu que le dédoublement obéit à la formule de réaction monomoléculaire.

[1]) Biochemische Zeitschrift, **66**, 344 (1914).

Toutefois cela n'est vrai qu'autant que la concentration de l'enzyme par rapport à celle du corps à scinder est assez grande pour que l'autodigestion de l'enzyme puisse être négligée; si la concentration enzymatique est faible, la scission se termine à une vitesse plus faible que celle correspondant à la formule monomoléculaire.

La dipeptide exerce une action protectrice sur l'enzyme, et l'autodigestion de celle-ci dépend manifestement de la concentration du corps à scinder.

Le produit du dédoublement, la glycocolle, pas plus que les ions K^+, Na^+, Cl^-, Br^-, F^-, SO^{--}_4 ou NO^-_3, en solutions étendues n'exerce aucune influence sur la vitesse de réaction; l'ion ClO^-_3, au contraire, tend à entraver l'action enzymatique, et l'ion CN^- joue le rôle d'un fort poison.

La première partie des recherches que je viens de relater a été exécutée au Laboratoire de Carlsberg. Je me fais un devoir de présenter encore ici au chef de cet institut, M. le Professeur S. P. L. Sörensen, l'hommage de ma vive gratitude pour l'hospitalité qu'il m'a témoignée et pour les bienveillantes instructions qu'il m'a prodiguées. Je dois également mes sincères remerciements à M. Hans Jessen-Hansen et à M. S. Palitzsch, attachés au Laboratoire, pour l'amiable concours qu'ils m'ont prêté.

La seconde partie de ce travail a été effectuée à l'Institut Nobel pour la chimie générale, à Stockholm, dont le directeur, M. le Professeur S. Arrhenius, mérite toute ma reconnaissance pour les précieux conseils dont il a bien voulu m'aider.

Institut Nobel, à Stockholm, le 1er novembre 1915.

RECHERCHES SUR LES ORGANISMES DE FERMENTATION.

PAR

ALB. KLØCKER.

III.
OBSERVATIONS RELATIVES A LA CONSERVATION D'ORGANISMES DE FERMENTATION DANS DES MILIEUX NUTRITIFS.

1. Introduction.

La présente étude se rattache en plusieurs points, pour y faire suite, aux recherches publiées en 1898 par E. Chr. Hansen[1]), en ce sens qu'elle porte en grande partie sur les végétations mêmes examinées par lui à cette époque-là.

Il a été constaté de nouveau que la conservation des levures et des moisissures dans une solution aqueuse à 10 pour cent de saccharose constitue une méthode excellente. Cependant, l'aperçu donné ci-dessous fera voir que, même cultivés dans le moût de bière, ces champignons peuvent se maintenir en vie pendant bien longtemps; pour ce qui concerne certaines formes — comme, par exemple, le Saccharomycodes Ludwigii et des espèces de Schizosaccharomyces —, ce dernier liquide doit même être considéré comme mieux approprié que la solution de saccharose.

Par saccharose et dextrose nous entendons dans ce mémoire des solutions aqueuses à 10 % de ces sucres, de même que par «eau de levure à saccharose» et «eau de levure à dextrose» nous désignons leurs solutions à 10 % dans l'eau de levure. Le moût employé était du moût de bière de garde de 13 à 14 % Balling, lequel cependant, dans presque tous les cas, s'est naturellement, au cours de la conservation, converti en bière. Les gélatines utilisées sont à 8—10 %. Enfin, le riz employé avait dans chaque cas été stérilisé en l'état humide, à la vapeur.

[1]) E. Chr. Hansen: Sur la vitalité des ferments alcooliques et leur variation dans les milieux nutritifs et à l'état sec. (Comptes-rendus du Laboratoire de Carlsberg, **4**, 93 (1898)).

Les végétations que nous qualifierons de mortes, ne comprennent pas celles — peu nombreuses d'ailleurs —, qui, par suite de l'évaporation du liquide, se sont desséchées avec le temps et qui de ce fait ont péri; car ceci tient à des conditions anormales et ne peut déroger en rien à l'utilité du liquide comme milieu de conservation. On évite l'évaporation en se servant des ballons Pasteur, qui sont sans conteste les meilleurs lorsqu'il s'agit d'une conservation prolongée. Leur emploi comporte pourtant ce double inconvénient, qu'ils tiennent beaucoup de place, et que les tubes de caoutchouc sont aptes à se durcir à la longue et se brisent alors facilement; on peut y remédier en fermant le tube latéral avec un bouchon d'asbeste et cachetant avec de la cire. Une grande partie des végétations conservées dans le Laboratoire de Carlsberg le sont dans des flacons Freudenreich dont, afin de réduire l'évaporation, le tube du capuchon est pourvu, à l'aide d'un petit tube de caoutchouc, d'un tube capillaire courbé en forme d'une S, dispositif qui dans la plupart des cas s'est trouvé bien efficace[1]).

D'une façon générale, on peut dire qu'il vaut mieux conserver les cultures dans une solution de saccharose que dans du moût de bière, vu que le premier de ces deux liquides présente une constitution plus homogène que le second, dont la composition toujours plus ou moins variable semble susceptible d'avoir une influence néfaste sur la vie des végétations. Effectivement, il arrive parfois que les organismes périssent subitement dans le moût, sans qu'il soit possible d'en découvrir la cause. C'est ainsi que dans la suite il sera fait mention de trois cas dans chacun desquels un Saccharomyces a succombé dans du moût avant l'expiration de 2 mois, alors que les autres végétations, dont le semis avait été effectué simultanément, se sont maintenues vivantes; encore ces trois végétations-là provenaient-elles directement de trois des plus anciennes cultures en moût, vieilles respectivement de 28, 27½ et 26 ans. Nous ne saurions chercher l'explication de ces faits que dans la composition défavorable du moût employé comme milieu de culture.

Sauf pour les bactéries d'acide acétique, nous n'avons pas compris dans ces recherches un grand nombre de végétations conservées dans notre laboratoire depuis 5 ans seulement.

[1]) Un flacon Freudenreich muni de ce tube se trouve figuré dans Alb. Kløcker: Die Gärungsorganismen in der Theorie und Praxis der Alkoholgärungsgewerbe, 2. Ausgabe, 1906, p. 50, Fig. 53.

Il est d'une importance capitale de pouvoir conserver les différentes espèces durant une longue suite d'années. Quant à la question de savoir comment il faut procéder à cet effet, on ne saurait la trancher à priori: l'expérience seule peut en décider. C'est pourquoi je crois utile de faire connaître ici les résultats que nous avons pu tirer des expériences puisées pendant plus de trente années au Laboratoire de Carlsberg, qui, on le sait, fut le premier à préparer de vraies cultures pures de levures et où, par conséquent, se trouvent les végétations les plus anciennes.

En ce qui concerne bon nombre des végétations trouvées mortes, nous pouvons seulement indiquer qu'elles ont péri avant l'expiration de tel ou tel nombre d'années, ce qui, vu qu'aucune épreuve n'a été faite à un moment antérieur pour vérifier si ces cultures étaient restées en vie, implique évidemment qu'elles ont pu succomber longtemps avant le jour où leur mort a été constatée.

Parmi les communications publiées dans ces dernières années au sujet de la conservation de microorganismes dans des liquides nutritifs, il faut citer ici celles qui suivent:

H. Will (Centralbl. f. Bakt. etc. 2. Abt., 24, 405 (1909)) a observé que, dans des solutions à 10 % de saccharose, une levure de vin s'était conservée en vie durant $9^1/_4$ années, une levure de bière à fermentation basse pendant $7^3/_4$ ans, et 9 autres levures de 5 à $6^3/_4$ ans. Dans le même liquide nutritif, une levure de vin était morte au bout de $3^1/_4$ ans, un Saccharomyces Pastorianus après $2^3/_4$ ans, une levure à fermentation haute après $2^1/_2$ ans, et 2 levures de bière à fermentation basse après $1^1/_4$ an. W. Bierberg (Ber. d. K. Lehranstalt f. Wein-, Obst- und Gartenbau in Geisenheim für 1909), qui avait conservé depuis 10 à 11 ans 101 levures de vin dans des solutions de saccharose, constata que 9 d'entre elles s'étaient tout à fait desséchées. et que parmi toutes les autres, au nombre de 92, 4 seulement avaient cessé de vivre. R. Meissner (Zeitschr. f. Gärungsphysiologie, **I**, 106 (1912)) trouva que, sur 25 différentes levures de vin conservées dans des solutions à 10 % de saccharose, 15 étaient encore vivantes après $10^1/_4$ ans, tandis que 9 avaient péri avant l'expiration de $8^1/_4$ ans, et qu'une seule, trouvée encore en vie après $8^1/_4$ ans, l'avait perdue au bout de $10^1/_4$ ans. — Ainsi qu'on le verra par la suite, aucune des végétations de levure de vin conservées dans le Laboratoire de Carlsberg n'était morte après 10 à 30 années de séjour soit dans de la saccharose, soit dans du moût. Enfin, nous

pouvons citer que, sur 11 végétations de levure basse de bière conservées par H. Will (Centralbl. f. Bakt. etc. 2. Abt., **44**, 58 (1915)) dans des ballons Pasteur renfermant du moût, une avait atteint l'âge de $18^1/_6$ ans, et que 6 dataient de 17 ans et quelques mois, une de $16^5/_6$ ans, l'âge les autres allant de $11^1/_6$ à $16^1/_4$ ans.

2. Levures et Moisissures.

J'ai soumis, en tout, 820 végétations à l'épreuve relative à la vie.

Elles étaient formées des organismes énumérés ci-après:

581 Saccharomycètes. 36 Schizosaccharomycètes. 35 Formes de Torula. 1 Mycoderma (cerevisiae). 7 Endomyces. 11 Monilia. 6 Chalara. 14 Oïdium. 43 Mucoracées. 86 autres Moisissures.

Les 581 Saccharomycètes appartenaient aux genres suivants:

422 Saccharomyces. 46 Variétés asporogènes de Saccharomyces. 18 Saccharomycodes (Ludwigii). 16 Zygosaccharomyces. 24 Saccharomycopsis (capsularis). 27 Willia. 23 Pichia. 5 Hanseniaspora.

Les 422 Saccharomyces étaient les suivants:

17 Sacch. cerevisiae. 33 autres levures de bière à fermentation haute. 18 Levures de distillerie et levures employées dans la fabrication de levure pressée. 20 Sacch. validus. 29 S. intermedius. 12 autres levures à fermentation basse. 17 Sacch. Carlsbergensis. 17 S. Monacensis. 60 autres levures de bière à fermentation basse. 21 Sacch. ellipsoïdeus. 24 Johannisberg II. 29 autres levures de vin. 17 Sacch. Pastorianus. 23 S. turbidans. 1 S. Brasiliensis. 2 S. Hansenii. 10 S. exiguus. 6 S. Marxianus. 4 S. lactis. 1 S. fragilis. 61 Levures sauvages.

Les 36 Schizosaccharomyces étaient les suivants:

16 Schizosacch. octosporus. 6 S. Pombe. 14 S. sp.

Les 35 Torula étaient:

17 Torula sp 10 Pseudosaccharomyces apiculatus. 7 Ps. sp. 1 Torula rouge.

Les 14 Oïdium:

2 Oïdium lactis. 2 O. lupuli. 10 O. sp.

Les 43 Mucoracées:

4 Mucor Mucedo. 6 M. racemosus. 2 M. rhizopodiformis. 3 M. erectus. 4 M. spinosus. 18 M. sp. 2 Sporodinia grandis. 4 Amylomyces Rouxii.

Les autres moisissures, au nombre de 86, appartenaient aux genres que voici: 23 Penicillium. 2 Citromyces. 36 Aspergillus. 1 Boudiera. 2 Eurotiopsis. 20 Botrytis. 1 Cladosporium. 1 Dematium.

Les 23 Penicillium étaient: 4 Penicil. glaucum. 9 P. luteum. 2 P. Wortmanni. 8 P. sp.

Les 36 Aspergillus: 6 Asp. glaucus. 2 A. repens. 3 A. fumigatus. 4 A. oryzae. 1 A. flavus. 2 A. flavescens. 1 A. niger. 2 A. clavatus. 2 A. nidulans. 13 A. sp.

On trouvera ci-dessous, au Tableau I, un aperçu du nombre des végétations vivantes ou mortes, conservées dans les divers milieux nutritifs.

TABLEAU I.

	En saccharose		En moût		Dans d'autres milieux	
	vivantes	mortes	vivantes	mortes	vivantes	mortes
Levures et moisissures	403	58	268	22	54	15
Saccharomycètes	270	40	220	20	21	10
Saccharomyces	219	12	181	9	1	0
Sacch. cerevisiae	9	0	6	2	»	»
D'autres levures hautes de bière	20	4	9	0	»	»
Lev. de distil. et de fabr. de lev. pres.	12	1	3	2	»	»
Sacch. validus	4	1	14	1	»	»
Sacch. intermedius	10	0	18	1	»	»
D'autres levures hautes	8	0	4	0	»	»
Sacch. Carlsbergensis	3	0	13	1	»	»
Sacch. Monacensis	8	0	9	0	»	»
D'autres levures basses de bière	29	0	31	0	»	»
Sacch. ellipsoïdeus	8	0	13	0	»	»
Johannisberg II	17	0	7	0	»	»
D'autres levures de vin	23	0	6	0	»	»
Sacch. Pastorianus	9	0	6	2	»	»
Sacch. turbidans	7	0	16	0	»	»
Sacch. Brasiliensis	1	0	»	»	»	»
Sacch. Hansenii	»	»	1	0	1[1])	0
Sacch. exiguus	4	1	5	0	»	»
Sacch. Marxianus	3	0	3	0	»	»
Sacch. lactis	2	0	2	0	»	»
Sacch. fragilis	»	»	1	0	»	»
Levures sauvages	42	5	14	0	»	»
Variétés asporogènes de Sacch.	14	18	3	11	»	»
Saccharomycodes Ludwigii	1	8	5	0	»	»
Zygosaccharomyces	5	0	9	0	2[2])	0
Saccharomycopsis capsularis	2	2	2	0	12[3])	6[4])

[1]) En eau de levure + dextrose. [2]) Eau de levure + dextrose. [3]) Saccharose. [3]) Eau de levure. [3]) Décoction de foin. [3]) Riz. [3]) Moût de raisins + gélatine + agar. [3–4]) Eau de levure + gélatine. [4]) Eau de levure + gélatine + agar. [4]) Moût de bière gélatiné. [4]) Moût gélatiné additionné d'agar.

Tableau I (Suite).

	En saccharose		En moût		Dans d'autres milieux	
	vivantes	mortes	vivantes	mortes	vivantes	mortes
Willia	15	0	9	0	2	1
W. anomala	3	0	1	0	»	»
W. Saturnus	4	0	1	0	2 [1]	1 [2]
W. sp.	8	0	7	0	»	»
Pichia	11	0	9	0	3	0
P. membranaefaciens	3	0	8	0	3 [3]	0
Pichia sp.	8	0	1	0	»	»
Hanseniaspora	3	0	2	0	»	»
Schizosaccharomyces	2	3	10	1	17	3
Sch. octosporus	1	0	3	1	11 [4]	0
Sch. Pombe	0	1	2	0	2 [5]	1 [6]
Schizosacch. sp.	1	2	5	0	4 [7]	2 [7]
Formes de Torula	7	1	23	0	3	1
Torula	5	0	11	0	1 [8]	0
Pseudosaccharomyces apiculatus	1	1	5	0	2 [9]	1 [10]
Ps. sp.	»	»	7	0	»	»
Torula rouge	1	»	»	»	»	»
Mycoderma cerevisiae	»	»	1	0	»	»
Endomyces sp.	1	0	»	»	»	»
E. Javanensis	»	»	6	0	»	»
Monilia candida	6	1	0	1	2 [11]	0
M. sp.	»	»	»	»	1 [12]	»
Chalara	3	0	2	0	1 [13]	0
Oïdium	10	1	2	0	1	0
O. lactis	2	0	»	»	»	»
O. lupuli	2	0	»	»	»	»
O. sp.	6	1	2	0	1 [14]	0
Mucoracées	33	6	»	»	4	0
Mucor Mucedo	2	2	»	»	»	»
M. racemosus	4	1	»	»	1 [15]	»
M. rhizopodiformis	1	1	»	»	»	»
M. erectus	3	0	»	»	»	»
M. spinosus	2	0	»	»	2 [16]	0
M. sp.	15	2	»	»	1 [17]	0
Sporodinia grandis	2	0	»	»	»	»
Amylomyces Rouxii	4	0	»	»	»	»
D'autres moisissures	74	6	1	0	4	1
Penicillium	19	1	»	»	2	1
P. glaucum	4	0	»	»	»	»
P. luteum	6	1	»	»	1 [18]	1 [18]

[1]) Eau de levure + maltose. [2]) Eau de levure + dextrose. [3]) Eau de levure + dextrose. [4]) Riz, moût de bière gélatiné. [5]) Riz. [6]) Moût gélatiné. [7]) Riz. [8]) Eau de levure + dextrose. [9]) Solution de dextrose en eau et moût + dextrose. [10]) Eau. [11]) Eau de levure + saccharose. [12]) Eau de levure + lactose. [13]) Moût + acide tartrique. [14]) Eau de levure + saccharose. [15]) Eau de levure + sacch. [16]) Eau de levure + sacch. [17]) Eau de levure + sacch. [18]) Eau de levure + sacch.

Tableau I (Suite).

	En saccharose		En moût		Dans d'autres milieux	
	vivantes	mortes	vivantes	mortes	vivantes	mortes
P. Wortmanni	2	0	»	»	»	»
P. sp.	7	0	»	»	1 [1])	0
Citromyces sp.	2	0	»	»	»	»
Aspergillus	29	5	»	»	2	0
A. glaucus	1	5	»	»	»	»
A. repens	2	0	»	»	»	»
A. fumigatus	3	0	»	»	»	»
A. oryzae	4	0	»	»	»	»
A. flavus	1	0	»	»	»	»
A. flavescens	1	0	»	»	1 [2])	0
A. niger	1	0	»	»	»	»
A. clavatus	2	0	»	»	»	»
A. nidulans	1	0	»	»	1 [3])	0
A. sp.	13	0	»	»	»	»
Boudiera Clauseni	1	0	»	»	»	»
Eurotiopsis Gayoni	1	0	1	0	»	»
Formes de Botrytis	20	0	»	»	»	»
Cladosporium butyri	1	0	»	»	»	»
Dematium sp.	1	0	»	»	»	»

[1]) Eau de levure + saccharose. [2]) Eau de levure + saccharose. [3]) Eau de levure + saccharose.

Le Tableau II fait voir le nombre des végétations qui s'étaient maintenues vivantes dans de la saccharose, ainsi que la durée de leur vie; le Tableau III, le nombre des végétations mortes dans la saccharose; le Tableau IV, les végétations encore vivantes dans du moût, et le Tableau V, celles mortes dans ce liquide. Enfin, le Tableau VI comprend les végétations conservées vivantes dans d'autres milieux, et le Tableau VII celles qui y étaient mortes.

TABLEAU II.

	Vivant en saccharose					
	entre 5 et 10 ans	entre 10 et 15 ans	entre 15 et 20 ans	entre 20 et 25 ans	entre 25 et 30 ans	plus de 30 ans
Saccharomyces	47	35	67	18	46	6
Sacch. cerevisiae	1	»	4	1	3	»
D'autres levures hautes de bière	15	1	3	»	»	1
Lev. de destil. et de fabr. de lev. pres.	1	4	4	»	3	»
Sacch. validus	1	»	»	1	2	»
Sacch. intermedius	1	»	6	1	2	»

Tableau II (Suite).

	Vivant en saccharose					
	entre 5 et 10 ans	entre 10 et 15 ans	entre 15 et 20 ans	entre 20 et 25 ans	entre 25 et 30 ans	plus de 30 ans
D'autres levures hautes	2	4	»	»	2	»
Sacch. Carlsbergensis	»	»	2	»	»	1
Sacch. Monacensis.....	»	»	7	»	1	»
D'autres levures basses de bière	2	5	7	5	10	»
Sacch. ellipsoïdeus	»	»	4	1	2	1
Johannisberg II	3	1	13	»	»	»
D'autres levures de vin	7	4	12	»	»	»
Sacch. Pastorianus.....	1	»	4	2	2	»
Sacch. turbidans	1	»	»	1	5	»
Sacch. Brasiliensis.....	»	1	»	»	»	»
Sacch. exiguus.....	»	»	»	»	4	»
Sacch. Marxianus	»	»	»	»	3	»
Sacch. lactis.....	»	»	»	2	»	»
Levures sauvages.....	12	15	1	4	7	3
Variétés aspor. de Saccharom.	3	»	11	»	»	»
Saccharomycodes Ludwigii...	1	»	»	»	»	»
Zygosaccharomyces	1	4	»	»	»	»
Saccharomycopsis capsularis..	2	»	»	»	»	»
Willia	2	11	2	»	»	»
W. anomala	1	»	2	»	»	»
W. Saturnus.....	»	4	»	»	»	»
Willia sp.....	1	7	»	»	»	»
Pichia	7	2	»	»	2	»
P. membranaefaciens.....	1	»	»	»	2	»
Pichia sp.....	6	2	»	»	»	»
Hanseniaspora	»	3	»	»	»	»
Schizosaccharomyces	»	1	1	»	»	»
Sch. octosporus	»	»	1	»	»	»
Sch. sp.	»	1	»	»	»	»
Formes de Torula	»	2	»	»	5	»
Torula	»	2	»	»	3	»
Pseudosacch. apiculatus	»	»	»	»	1	»
Torula rouge	»	»	»	»	1	»
Endomyces sp.....	»	»	1	»	»	»
Monilia candida.....	»	»	3	»	3	»

Tableau II (Suite).

	Vivant en saccharose					
	entre 5 et 10 ans	entre 10 et 15 ans	entre 15 et 20 ans	entre 20 et 25 ans	entre 25 et 30 ans	plus de 30 ans
Chalara	1	2	»	»	»	»
Oïdium lactis	»	2	»	»	»	»
O. lupuli	2	»	»	»	»	»
O. sp.	5	»	1	»	»	»
Mucoracées	13	17	1	»	2	»
Mucor Mucedo	2	»	»	»	»	»
M. racemosus	2	1	1	»	»	»
M. rhizopodiformis	1	»	»	»	»	»
M. erectus	1	1	»	»	1	»
M. spinosus	»	1	»	»	1	»
M. sp.	2	13	»	»	»	»
Sporodinia grandis	2	»	»	»	»	»
Amylomyces Rouxii	3	1	»	»	»	»
Penicillium	9	7	3	»	»	»
P. glaucum	»	2	2	»	»	»
P. luteum	6	»	»	»	»	»
P. Wortmanni	»	2	»	»	»	»
P. sp.	3	3	1	»	»	»
Citromyces sp.	2	»	»	»	»	»
Aspergillus	10	5	12	»	2	»
A. glaucus	1 [1])	»	»	»	»	»
A. repens	»	1	1	»	»	»
A. fumigatus	»	»	2	»	1	»
A. oryzæ	»	1	3	»	»	»
A. flavus	»	»	1	»	»	»
A. flavescens	»	»	1	»	»	»
A. niger	»	»	»	»	1	»
A. clavatus	2	»	»	»	»	»
A. nidulans	»	»	1	»	»	»
A. sp.	7	3	3	»	»	»
Boudiera Clauseni	1	»	»	»	»	»
Eurotiopsis Gayoni	1	»	»	»	»	»
Formes de Botrytis	20	»	»	»	»	»
Cladosporium butyri	»	1	»	»	»	»
Dematium sp.	1	»	»	»	»	»

[1]) 2 ans.

TABLEAU III.

	Mortes, en saccharose																						
	avant l'expiration de — ans																entre — et — ans						
	2	3	6	7 1/2	8	9 1/2	10	10 1/2	11	12 1/2	16	16 1/2	17 1/2	18	18 1/2	20	6 et 10	10 et 16	19 et 25	20 et 27	22 et 28	23 et 29	25 et 31
Saccharomyces	»	»	1	2	»	1	»	»	»	1	»	1	1	»	»	»	»	»	1	1	»	2	1
Levures hautes de bière	»	»	1	»	»	1	»	»	»	1	»	»	1	»	»	»	»	»	»	»	»	»	»
Levures de distillerie et de fabrication de levure pressée	»	»	»	»	»	»	»	»	»	»	»	1	»	»	»	»	»	»	»	»	»	»	»
Sacch. validus	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	1	»	»	»
Sacch. exiguus	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	1	»	»	»	»
Levures sauvages	»	»	»	2	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	2	1
Variétés asporog. de Sacch.	»	1	3	»	»	»	»	»	»	»	»	»	»	»	1	»	5	8	»	»	»	»	»
Saccharomycodes Ludwigii	»	2	5	1	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
Saccharomycopsis capsularis	»	»	»	»	»	»	2	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
Schizosaccharomyces	»	»	»	»	»	»	3	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
Sch. Pombe	»	»	»	»	»	»	1	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
Sch. sp.	»	»	»	»	»	»	2	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
Pseudosacch. apiculatus	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	1	»	»
Monilia candida	»	»	»	»	»	»	»	»	»	»	1	»	»	»	»	»	»	»	»	»	»	»	»
Oïdium sp.	»	»	»	»	»	1	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
Mucoracées	»	»	»	1	»	»	1	2	1	»	»	»	»	1	»	»	»	»	»	»	»	»	»
Mucor Mucedo	»	»	»	»	»	»	1	»	1	»	»	»	»	»	»	»	»	»	»	»	»	»	»
M. racemosus	»	»	»	1	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
M. rhizopodiformis	»	»	»	»	»	»	»	»	»	»	»	»	»	1	»	»	»	»	»	»	»	»	»
Mucor sp.	»	»	»	»	»	»	»	2	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»
Penicillium luteum	»	»	»	»	»	»	»	»	»	»	»	»	»	»	»	1	»	»	»	»	»	»	»
Asp. glaucus	2	»	»	»	1	1	»	»	»	»	1	»	»	»	»	»	»	»	»	»	»	»	»

TABLEAU IV.

	Vivant en moût de bière				
	entre 5 et 10 ans	entre 10 et 15 ans	entre 15 et 20 ans	entre 20 et 25 ans	entre 25 et 30 ans
Saccharomyces	17	25	63	12	64
Sacch. cerevisiae	»	»	3	»	3
D'autres lev. hautes de bière	7	2	»	»	»
Levures de distillerie et de fabrication de lev. pressée	1	2	»	»	»
Sacch. validus	»	»	2	»	12
Sacch. intermedius	»	»	3	»	15
D'autres levures hautes	»	4	»	»	»
Sacch. Carlsbergensis	»	»	10	3	»
Sacch. Monacensis	»	»	8	1	»
D'autres lev. basses de bière	2	3	22	4	»
Sacch. ellipsoïdeus	»	»	2	»	11
Johannisberg II	»	»	7	»	»
D'autres levures de vin	4	2	»	»	»
Sacch. Pastorianus	»	»	2	»	4
Sacch. turbidans	»	»	2	»	14
Sacch. Hansenii	»	»	»	1	»
Sacch. exiguus	»	»	»	2	3
Sacch. Marxianus	»	»	2	»	1
Sacch. lactis	»	1	»	1	»
Sacch. fragilis	1	»	»	»	»
Levures sauvages	2	11	»	»	1
Variétés aspor. de Sacchar.	1	»	2	»	»
Saccharomycodes Ludwigii.	»	»	2	3	»
Zygosaccharomyces	2	7	»	»	»
Saccharomycopsis capsularis	2	»	»	»	»
Willia	1	7	1	»	»
W. anomala	»	»	1	»	»
W. Saturnus	»	1	»	»	»
Willia sp.	1	6	»	»	»
Pichia	1	2	1	1	4
P. membranaefaciens	1	»	2	1	4
Pichia sp.	»	1	»	»	»
Hanseniaspora	»	2	»	»	»
Schizosaccharomyces	7	3	»	»	»
Sch. octosporus	3	»	»	»	»
Sch. Pombe	1	1	»	»	»
Sch. sp.	3	2	»	»	»
Formes de Torula	»	13	2	3	5
Torula	»	6	»	2	3
Pseudosacch. apiculatus	»	»	2	1	2
Ps. sp.	»	7	»	»	»
Mycoderma cerevisiae	»	»	»	»	1
Endomyces Javanensis	6	»	»	»	»
Chalara	1	1	»	»	»
Oïdium sp.	2	»	»	»	»
Eurotiopsis Gayoni	1	»	»	»	»

TABLEAU V.

	Mortes en moût								
	avant l'expiration de — ans							entre — et — ans	
	1/6	1/3	3	6	12	15	27	3 et 6	6 et 10
Saccharomyces	3	2	»	»	»	3	1	»	»
Sacch. cerevisiae	1	»	»	»	»	»	1	»	»
Levures de distillerie et de fabrication de levure pressée	»	2	»	»	»	»	»	»	»
Sacch. validus	1	»	»	»	»	»	»	»	»
Sacch. intermedius	1	»	»	»	»	»	»	»	»
Sacch. Carlsbergensis	»	»	»	»	»	1	»	»	»
Sacch. Pastorianus	»	»	»	»	»	2	»	»	»
Variétés asporogène de Sacch.	»	»	6	1	»	»	»	3	1
Schizosacch. octosporus	»	»	»	1	»	»	»	»	»
Monilia candida	»	»	»	»	1	»	»	»	»

TABLEAU VI.

	Entre 5 et 10 ans	Entre 10 et 15 ans	Entre 15 et 20 ans	Entre 20 et 25 ans	Entre 25 et 30 ans
	Vivant en eau de levure additionnée de dextrose				
Sacch. Hansenii	»	»	»	1	»
Saccharomycodes Ludwigii	»	»	»	1	»
Zygosacch. sp.	»	2	»	»	»
Pichia membranaefaciens	»	»	»	2	1
Torula	»	»	»	1	»
	En solution aqueuse de dextrose				
Pseudosacch. apiculatus	»	»	»	»	1
	En eau de levure additionnée de saccharose				
Saccharomycopsis capsularis	1	»	»	»	»
Monilia candida	»	»	1	1	»
Oïdium sp.	»	»	»	»	1
Mucor racemosus	»	»	»	1	»
M. spinosus	»	»	»	»	2
M. sp.	»	»	»	»	1
Penicillium luteum	»	»	»	1	»
P. sp.	»	»	»	»	1
Aspergillus flavescens	»	»	»	»	1
A. nidulans	»	»	»	»	1
	En eau de levure				
Saccharomycopsis capsularis	4	2	»	»	»
	En eau de levure additionnée de maltose				
Willia Saturnus	1	1	»	»	»

Tableau VI (Suite).

	Entre 5 et 10 ans	Entre 10 et 15 ans	Entre 15 et 20 ans	Entre 20 et 25 ans	Entre 25 et 30 ans
	Eau de levure additionnée de lactose				
Monilia sp.	»	»	»	1	»
	Moût de bière + dextrose				
Pseudosacch. apiculatus	»	»	»	»	1
	Moût + acide tartrique				
Chalara	»	1	»	»	»
	Décoction de foin				
Saccharomycopsis capsularis	1	»	»	»	1
	Moût gélatiné				
Schizosacch. octosporus	4	2	»	»	»
	Eau de levure + gélatine				
Saccharomycopsis capsularis	1 [1]	»	»	»	»
	Moût de raisins + gélatine				
Saccharomycopsis capsularis	1	»	»	»	»
	Riz				
Saccharomycopsis capsularis	1	»	»	»	»
Schizosacch. octosporus	5	»	»	»	»
Schizosacch. Pombe	2	»	»	»	»
Schizosacch. sp.	4	»	»	»	»

[1]) et 1 en $2^3/_4$ ans.

TABLEAU VII.

	Mortes avant l'expiration de — ans					Mortes entre — et — ans		
	$2^3/_4$	6	$8^1/_2$	$8^3/_4$	9	$13^3/_4$ et 20	15 et $21^1/_2$	$20^1/_4$ et $26^1/_4$
	Eau de levure + dextrose							
Sacch. Ludwigii	»	»	»	»	»	1	2	»
Willia Saturnus	»	»	»	»	1	»	»	»
	Eau de levure + saccharose							
Penicil. luteum	»	»	»	»	»	1	»	»
	Eau							
Pseudosacch. apiculatus	»	»	»	»	»	»	»	1
	Moût gélatiné							
Saccharomycopsis capsularis	»	»	»	1	»	»	»	»
Schizosacch. Pombe	»	1	»	»	»	»	»	»
	Eau de levure + gélatine							
Saccharomycopsis capsularis	1	»	»	»	»	»	»	»

Tableau VII (Suite).

	Mortes avant l'expiration de — ans					Mortes entre — et — ans		
	$2^3/_4$	6	$8^1/_2$	$8^3/_4$	9	$13^3/_4$ et 20	15 et $21^1/_2$	$20^1/_4$ et $26^1/_4$
	Moût gélatine additionné d'agar							
Saccharomycopsis capsularis..	»	»	»	»	3	»	»	»
	Eau de levure + gélatine + agar							
Saccharomycopsis capsularis..	»	»	1	»	»	»	»	»
	Riz							
Schizosacch. sp.....	»	1	1	»	»	»	»	»

3. Bactéries d'acide acétique.

On a soumis à l'épreuve, en tout, 31 végétations, et les a trouvées toutes en vie. C'étaient les espèces suivantes:

10 Bacterium aceti.
12 — Pasteurianum.
9 — Kützingianum.

Les résultats sont consignés au Tableau VIII.

TABLEAU VIII.

	Vivant en double bière					Bière de garde + gélatine	Double bière + gélatine
	$2^3/_4$ ans	$3^1/_4$ ans	$4^3/_4$ ans	$5^1/_2$ ans	$9^3/_4$ ans	13 ans	13 ans
Bact. aceti.....	2	2	1	2	3	»	»
Bact. Pasteurianum ..	3	3	1	3	»	1	1
Bact. Kützingianum .	3	3	»	3	»	»	»

Résumé.

Nous avons soumis à l'épreuve relative à la vie 820 végétations de levures et de moisissures, dont 461 se trouvaient dans une solution à 10 % de saccharose, 290 dans du moût de bière, et 69 dans d'autres milieux. Dans la saccharose, 403 végétations (soit 87 %) se sont trouvées avoir conservé la vie, tandis que 58 (13 %) l'avaient perdue; dans le moût, 268 (92 %) étaient vivantes, 22 (8 %) mortes. Aussi bien une solution aqueuse à

10 % de saccharose que du moût de bière de garde (13 à 14 % Ball.), se prêtent donc éminemment à la conservation de levures et de moisissures. Les organismes peuvent s'y maintenir en vie durant plus de 30 ans.

Font exception à cette règle:

1° Les variétés asporogènes de Saccharomyces:

Sur 32 cultures en saccharose, 14 (44 %) seulement étaient restées en vie, alors que 18 (56 %) avaient péri; et sur 14 cultures en moût, nous n'en avons trouvé que 3 vivantes, mais 11 (78,6 %) mortes. Parmi les végétations encore vivantes, les plus anciennes avaient atteint l'âge de 15 à 20 ans.

2° Saccharomycodes Ludwigii:

Sur 9 cultures en saccharose, une seule était encore vivante, tandis que 8 étaient mortes avant l'expiration de 3 à $7^1/_2$ ans. Les 5 cultures en moût s'étaient toutes maintenues en vie, et avaient atteint l'âge de 25 ans.

3° Schizosaccharomyces:

Sur 5 cultures en saccharose, 2 seulement étaient vivantes (1 Sch. octosporus datait de $17^3/_4$ ans, 1 Sch. sp. de 11 ans); au contraire, sur 11 cultures en moût, 10 étaient vivantes. Sur le riz, ces formes paraissent pouvoir se conserver en vie, au moins jusqu'à 9 ans.

4° Aspergillus glaucus:

Cette espèce s'est comportée d'une façon toute différente des autres espèces appartenant à ce genre. Sur 6 cultures en saccharose, une seule était vivante au bout de 2 ans, tandis que 2 autres étaient mortes avant l'expiration de ce terme.

Les trois bactéries d'acide acétique de Hansen peuvent vivre pendant 5—9 ans dans la double bière.

Laboratoire de Carlsberg, en janvier 1916.

SUR LA FORMATION D'UNE SUBSTANCE RESSEMBLANT A LA FLUORESCÉINE DANS LES CULTURES D'ASPERGILLUS GLAUCUS.

PAR

ALB. KLØCKER.

Il y a quelques années, j'ai remarqué qu'un moût de bière dans lequel croissait l'Aspergillus glaucus prenait peu à peu une teinte verdâtre fluorescente. Je n'ai guère tâché à cette époque-là d'approfondir ce phénomène; mais lorsque, il y a quelque temps, en versant dans de l'eau un peu de ce moût fluorescent, je la vis acquérir une fluorescence verte fortement prononcée, j'ai été amené à soumettre le phénomène à un examen plus attentif, qui a donné les résultats exposés ci-dessous.

Quand on cultive dans un liquide contenant du sucre l'Aspergillus glaucus (ou l'Asp. repens, espèce probablement identique à celle-là) et en même temps une autre forme de couleur verte et à formation de périthèces — forme qui paraît également appartenir à l'A. glaucus —, on voit se produire une substance qui rappelle la fluorescéine. M. Jessen-Hansen, chimiste attaché au Laboratoire de Carlsberg, a eu l'obligeance d'examiner, autant que cela a été possible, ce corps au point de vue de sa nature chimique; ce n'est pas la fluorescéine, mais un composé qui paraît s'y rattacher d'assez près.

Lorsqu'un pareil liquide sucré, où l'on a cultivé l'Asp. glaucus ou une des formes voisines, est agité en présence d'un égal volume d'éther, celui-ci prend une teinte jaunâtre, associée en couches épaisses avec une fluorescence bleue. Si ensuite on décante l'éther et qu'on le secoue avec de l'eau ammoniacale à 10 % et de même volume, on verra celle-ci acquérir une fluorescence verte très marquée. En remplaçant l'ammoniaque par la soude, on obtient une fluorescence rouge brun. Quand on évapore l'éther de la solution éthérique, il reste un résidu jaune, qui est facilement soluble dans l'eau. Cette solution aqueuse affecte

une faible fluorescence; mais si l'on y ajoute de la lessive de soude, le liquide se colore en rouge brun et montre une fluorescence verte très prononcée, qui pourtant se perd peu à peu à la longue. Quand on agite une solution éthérique avec de l'eau, celle-ci ne prend aucune coloration. Si l'on ajoute NaOH, la liqueur alcaline absorbe intégralement la matière colorante et devient rouge brun ou vineux. En substituant l'ammoniaque au NaOH, on observe une fluorescence bien marquée.

Quand on cultive l'Aspergillus, par exemple, sur du moût de bière gélatinisé, la gélatine liquéfiée prend également la fluorescence.

Abandonné au repos pendant quelques jours, le mélange d'éther et d'ammoniaque perd la fluorescence verte, et l'eau ammoniacale prend une teinte rougeâtre.

La réaction signalée ci-dessus est propre aux Asp. glaucus et repens, et peut même être utilisée comme caractère spécifique.

J'ai expérimenté encore plusieurs autres espèces de ce genre, notamment les A. oryzae et niger, et j'ai étendu ces recherches aux Penicillium glaucum et P. Wortmanni, Botrytis, Cladosporium, etc.; mais aucune d'elles n'a donné de fluorescence. Seule, une espèce de Penicillium a montré une légère fluorescence dans du moût de bière; il en a été de même lorsque le moût pur et le moût gélatiné liquéfié avaient subi un traitement par l'éther et l'eau ammoniacale.

Les faits que j'ai ainsi constatés ne se trouvent pas signalés dans la littérature qui m'a été accessible. A la vérité, il existe, comme l'on sait, des bactéries fluorescentes; mais le principe colorant qu'elles produisent est insoluble dans l'éther et, par conséquent, n'est pas identique à celui formé par l'Aspergillus (Flügge, Die Mikroorganismen, 3. Aufl. 1896. Tome II, page 290). E. Rostrup (Mykologiske Meddelelser, Bot. Tidskr. **17**, 1889, p. 233) a remarqué que l'Agaricus (Pleurotus) serotinus Schrad., placé dans l'esprit de vin, lui a communiqué une fluorescence particulière consistant en ce que par transparence le liquide présentait un beau reflet jaune d'or, tandis que par réflexion il montrait une couleur verte. De son côté, A. Ling (Journ. of the Inst. of Brewing, 1915, p. 528) a observé qu'un Torula se trouvant dans de l'Ale donne au liquide une fluorescence verdâtre.

Laboratoire de Carlsberg, en janvier 1916.

JOHS. SCHMIDT:

INVESTIGATIONS ON HOPS

(*Humulus lupulus*, L.).

IX. THE OCCURRENCE OF THE WILD HOP IN DENMARK.

WITH 1 CHART.

BY

JOHS. SCHMIDT.

I. Introduction.

For the furtherance of the work which is being carried on at the Carlsberg Laboratory, it was highly desirable to obtain fuller information as to the occurrence of the hop plant in Denmark than extant works afford. The floras make as a rule only brief mention of the hop, and nothing is stated with regard to its distribution throughout the country. In the herbaria also, it is but sparsely found, being apparently among the species rarely collected on botanical excursions.

With a plant so easily recognisable, however, and so generally known to others besides botanists, it seemed likely that reliable information might be obtained by sending out enquiries on the subject to various parts of the country.

I therefore, in 1911, had a number of enquiry forms printed, and these were then sent out to such persons in the different districts as might be presumed to have some knowledge of the questions concerned. The forms were addressed, for the most part, to forest officials and school teachers; also, where necessary, to others, as farmers, gardeners, etc.

The questions were as follows:

1. Is the wild hop common, rare, or altogether lacking in your district?
2. Is hop cultivation carried on in your district?
3. Have you any knowledge as to whether hop cultivation has formerly been carried on in your district?

4. Other remarks if any.
5. To what district (forest area, parish, jurisdiction, island, etc.) does your information apply? Kindly indicate this as accurately as possible.
6. If you are unable to answer the above questions, especially the first, to whom would you advise the Laboratory to apply for information regarding hops in your district?

The answers received were more numerous than had been expected, and within a couple of months, a sufficient amount of material had been procured for consideration of the question. There was also a surplus of printed forms, and these I consequently had sent to places abroad where Danish is understood by the inhabitants; i. e. Sweden, Norway, and the north of Slesvig. The information received from the Scandinavian peninsula will be dealt with later on, the present work including only the answers from Slesvig, which country is, of course, in geographical respects closely related to Denmark.

In Denmark and North Slesvig 570 copies of the enquiry were sent out. Of these, 503, or 88,3 %, were answered, a very satisfactory result. Many of the answers, moreover, contain information as to several districts.

The figures for the different divisions of the country are as follows:

	Enquiries sent	returned
Jutland with adjacent islands	220	190
Sealand (with the island of Møen etc.)	151	136
Funen (with Langeland etc.)	70	62
Lolland-Falster (with smaller islands)	33	30
Bornholm	9	8
[North Slesvig	87	77]

In addition to the statements received in answer to these enquiries, I have also had access to further information communicated by Danish botanists, or procured in the course of investigations carried out by the Carlsberg Laboratory.

I take this opportunity of cordially thanking the many persons — they number several hundred — whose kindly assistance alone has enabled me to carry out this work.

II. General remarks on the wild Hop.

It is not my intention here to give a detailed treatise on the biology of the wild hop, or the numerous forms in which this plant occurs in Denmark; to do so would require a far greater mass of observations than at present available. It will suffice to mention some of the more important features bearing on our present subject: to describe the occurence of the species in a wild state in this country.

The hop is a liana winding from left to right, which for its full developement requires a support, several metres high, up which it can wind. It also requires abundance of light, and protection against strong winds, and will not thrive in dark woods or in exposed, windy spots. It is by no means fastidious, however, as regards the nature of the soil, and may, as a matter of fact, be found growing in almost every kind of soil, save the most barren. The quantity of moisture in the ground is also of minor importance, and the hop may thrive as well in rather dry as in very damp earth.

The plant is perennial, the parts above ground dying off completely each year (in October and November); the underground portions of the stem consist of a short, vertical rootstock, which gradually becomes lignified, and attains a considerable thickness. From buds on this proceed those parts of the stem which appear above ground (in April); on reaching a certain height, they commence to wind («hop bines»). The lower, underground parts of the bine are thicker than the upper, and do not die off in autumn, but remain as branches from the rootstock. The root system is extremely strong, often penetrating to a great depth. The stronger roots are frequently much thickened towards the end, and may in some cases be said to be bulbous. The bulbous parts are mainly composed of parenchymatic tissue with starch-filled cells.

Vegetative propagation takes place by means of thin, horizontal side-shoots, running out from the root stock close beneath the surface of the soil and reaching a metre or more in length. In this manner, the same individual plant may in time extend over a large area, and the hop is thus, by the vegetative process alone, able to range over a considerable distance.

The hop is one of the few plants in our flora which are

dioecious; we may, however, quite exceptionally, find monoecious specimens. The species is typically anemophilous. The Laboratory has particularly studied the wild hop at one locality, to wit, Færgelunden near Frederikssund, North Sealand, where it is remarkably abundant in the copses and on the fringe of the alders close to Roskilde Fjord. Male and female plants are here found together, in about equal proportion. During the years from 1911—1915, flowering took place in August, the male plants commencing somewhat earlier than the female[1]). During all these years, ripe, fully developed seeds were produced. The observations for 1911 give the following particulars:

»By the 9th of July, many male plants were recognisable as such, but the flowers were as yet far from the opening stage. No female plants could be distinguished. By the 23rd July a great number of male plants had open and pollinating flowers, the majority, however, were not so far advanced. The pollinating plants were found only in spots open to the sun. On the 4th of August, the male plants were either in flower, or past the flowering stage. The females exhibited open flowers in sunny spots; not, however, as yet in shadier places. On the 11th of August, the male plants were more or less as before. In sunny spots, female plants were now found with hops 1—2½ cm. long. 14th August: Still many male flowers, many large hops. 18th August: Still a

[1]) In the floras I have found the following statements as to flowering time of the hop in Denmark.

Simon Paulli: Flora Danica, 1648, p. 273, states that it begins flowering on St. Mary Magdalen's Day.

Kylling: 1688, p. 88, mentions August as flowering time.

Rafn: 1800, p. 271, states that it flowers from end of June to beginning of August.

Schumacher: 1801, p. 291, gives June and July.

Hornemann: 1821, p. 1000, states that it flowers at the end of June.

Dreyer: 1838, p. 311, gives June and July.

Lange: 1886—88, p. 270, gives June and July as flowering time for the hop, and the same is stated by Raunkiær, 1914, p. 90.

As far as my own experience goes, the principal flowering time of the Danish hop is in the beginning of August, and I have not observed flowering to take place earlier than the latter half of July. There is, however, no reason to doubt that there exist in Denmark earlier-flowering forms than those which I have had an opportunity of observing, and the writers quoted above may therefore in part be right in stating an earlier time. We have, in our experimental nursery, plants, originally from England, which have flowered in the first half of June.

number of male plants in flower, especially in shady spots. 22nd August: The same. 25th August: Still some male plants in flower, but only in shady places. The hops now very large. 5th September: Flowering of male plants now over.«

Hop growers both in Denmark and other countries propagate their plants vegetatively by means of cuttings (i. e. parts of the underground stem). In this manner, a single individual can be divided up continually, and its life is thus prolonged indefinitely. There is no doubt that vegetative propagation is also of the greatest importance in the case of the wild hop. The seeds of the hop, as was shown by experiments made in 1911, germinate but slowly, and as the seedlings take a long time to develop, I was inclined to doubt whether seeding played any essential part in the propagation of the hop plants in the Danish climate. Investigations made the following year (1912), however, led to the finding, on the 15th May and 2nd June, of numerous young seedlings, first discovered by mag. sc. Ö. Winge, Assistant at the Laboratory. During the succeeding years (1913—15) the same has been observed each year. Thus it would seem that propagation by seeding takes place regularly every year, which fact is of considerable interest for the study of the biology of the wild hop in Denmark.

The young plants do not flower the first year, the earliest in the second, unless, as in our experimental nursery, special arrangements are made to induce rapid growth. By sowing the seeds under glass early in the year, planting out into a cold greenhouse, and carefully training the young seedlings up strings or sticks, we have succeeded each year in getting the majority of the plants to flower in their first summer.

The answers to the questions on the enquiry forms were as a rule but brief and of a summary nature. Several, however, especially from forest officials, furnished much information of great value as to the occurrence of the hop in the woods and forests of Denmark. These statements from different quarters agree extremely well in essentials, and afford, together with the results of the investigations carried out by the Laboratory, a very good idea as to the conditions under which the wild hop is found in this country.

I will now endeavour to give, in brief, the substance of the numerous observations taken as a whole. The individual com-

munications are, however, in some cases of so considerable interest in themselves that I cannot refrain from quoting portions word for word. Such extracts will be found on pp. 324—328, to which the reader may refer.

The hop is found in a wild state, (i. e. not cultivated), in woods, copses, hedgerows and the like. It is confined to such spots, both from its nature as a climbing plant, and also on account of the fact that it cannot thrive except where sheltered from the wind. At the same time, it requires a great deal of light, and consequently shuns the inner depths of close plantations, being found mostly on the fringe of the woods, in young copses, and more or less open spaces between trees, where there is abundance of light. The importance of this latter factor for the growth of the hop may be observed wherever the plants are seen on the outskirts of a wood; the plants will be found to be strongly developed at the very edge of the wooded ground, where the light is at its maximum, disappearing rapidly, however, as we pass in towards the deeper shade of the forest itself. Many of the forest officials state, moreover, that the hop is of frequent occurrence in young plantations of various sorts, but disappears as the trees grow up, and the plantation closes in.

By way of support, the hop may select the most widely differing kinds of plant, from a mere nettle *(Urtica)* or reed *(Phragmites)* to comparatively thick tree stems; there is a great difference, however, in the degree of frequency with which the various species are thus employed. Among our forest trees, the alder *(Alnus)*, ash *(Fraxinus)*, birch *(Betula)* and oak *(Quercus)* are those most often found acting as support for the hop; in the case of the oak and ash, young plantations are generally selected. It is rarely found growing up trees which give much shade, such as beech *(Fagus)* and spruce *(Picea)*, albeit this may, according to the information received, at times occur. It is frequently found among hazel *(Corylus)*, spindle tree *(Euonymus)*, buckthorn *(Rhamnus)* and other bushes of like dimensions.

III. Use of the wild Hop.

Generally speaking, the wild hop is not turned to any account. Here and there, however, probably for the most part in Funen, the hops from wild plants are gathered and dried, and

employed in the brewing of home-made beer, or offered for sale together with the crop of cultivated hops. Now and again, where the wild hop is found growing for instance on the edge of a wood, poles may be set to aid the growth, but no further care taken beyond this.

The value of the wild hop for brewing purposes is as a rule extremely poor, and that wild hops can be sold at all is due solely to the very low state of hop growing in this country, which may be estimated from the fact that Danish hops are sold here, not according to quality, but by quantity alone.

Some idea as to the amount of bitter resins to be found in wild hops may be gained from the following figures, from 1915, giving the total content of bitter resins in a number of different plants, the values being here shown as percentages of the total dry weight of the hops (cf. Winge & Jensen 1914):

Færgelunden, near Frederikssund, North Sealand.

6,0, 7,9, 6,4, 5,8, 8,1, 8,6, 5,8, 8,8 10,3, 6,7, 7,6, 9,4 9,8, 9,8 10,9, 13,8, 7,9, 12,1, 10,9, 7,9, 7,9, 7,8, 7,8, 8,1, 10,9, 11,4, 10,4.

Humlebæk, North Sealand.

6,3, 8,2, 7,8.

It will be seen from the figures, that the average content of bitter resins in all these plants is very poor; hardly more than half that of the German hops sold in this country. It is worthy of note, however, that some few plants show a bitter content far above the average. A percentage of 13,8 in a plant found growing among alders in a marshy patch, and entirely uncared for, is by no means insignificant, while the fact of a plant showing 13,8 % bitter resins being found among so small a number of specimens (30 in all) hints at the possibility of discovering, by extended investigations, others with a resin content sufficiently high to render their cultivation worth while.

IV. Occurrence of the wild Hop in different parts of the country.

In order to procure a survey as to the frequency of the wild hop[1]) in different parts of the country, I have made use of the information obtained from the answers to the enquiry forms sent out, in most cases without attempting to check the accuracy of

[1]) By »wild« is here understood »not cultivated«.

the statements in question. I have here endeavoured to arrange the material according to the following four categories; 1) »Common«, 2) »Found here and there«, 3) »Rare«, 4) »Not found«.

OCCURRENCE OF THE WILD HOP IN DENMARK.

Divisions of the country (The small islands not included)	No. of answers made use of	»Common«	»Here and there«	»Rare«	»Not found«
		%	%	%	%
Sealand (with Møen)	113	32	**47**	18	3
Funen (with Langeland) . . .	47	**70**	28	2	0
Lolland-Falster	26	**46**	**46**	8	0
Bornholm	11	9	**55**	9	27
Jutland (East)	149	11	**34**	25	30
— (West)	46	0	2	26	**72**

The result of this classification will be seen from the Table above and in the chart Pl. I. It need hardly be said that the classification here is, and must almost inevitably be, based upon personal estimate; the numerous statements received include a wide range of varying expressions in describing the frequency of the plants in the respective districts or areas. Another difficulty arises from the fact that the district referred to in one statement may not infrequently overlap that treated in another, involving some degree of repetition. The answers received, however, divide up the country pretty evenly, as will be seen from the chart Pl. I. As regards the first-mentioned difficulty, the personal element in the classification, there can after all be little doubt that the total impression produced by the statements when taken as a whole is correct, or approximately so, as may be seen, for instance from the fact that the different degrees of frequency are fairly evenly represented among the answers received, wherever the number is at all considerable.

As regards the different parts of the country, it will be seen from the Table that Funen is the region where wild hops are most frequently found (70 % »common«) the next in order being Lolland-Falster with 46 % »common«. Somewhat below this figure is Sealand (32 % »common«) as also Bornholm, so far as we can

judge from the few statements received from the last-named island. At the bottom of the list stands Jutland, though with considerable difference here between east and west, the plants being naturally more frequent in East Jutland (11 % »common«) than in the west[1]) (0 % »common«).

Selecting from the table, for each region, the category showing the highest percentage, we obtain the following:

Funen »common« (70 %).

Lolland-Falster: »common« and »here and there« (46 % and 46 %).

Sealand: »here and there« (47 %).

Bornholm: »here and there« (55 %).

East Jutland: »here and there« (34 %).

West Jutland: »not found« (72 %).

One feature should be pointed out; to wit, the fact that the wild hop appears to be lacking on some of the small islands, even those where general conditions would seem to favour its growth. This is the case, for instance, with several of the islands south of Funen. See also the chart Pl. I.

As regards the north of Slesvig, it will be seen from the chart that the wild hop, occurs regularly, especially in the eastern part of the country, whereas on the western side, according to the statements received, it is frequently lacking; i. e. much the same as noted in Danish Jutland.

It should be noted that the answers received frequently contained highly interesting information of so detailed a character as to preclude their inclusion in the present report.

Extracts from some of these, dealing with the occurrence of the hop in Danish forests, will be found on p. 324—328; for the rest, the answers received are preserved in the archives of the Laboratory, where they may be consulted by anyone subsequently wishing to take up the question.

The fact that the wild hop appears in varying degrees of frequency in the different parts of the country is of course primarily due to the differing natural conditions. In West Jutland, where the soil is poor, and the country open and exposed to the wind, the plants do not thrive, whereas regions such as for instance

[1]) Under West Jutland are included the administrative divisions of Ribe, Ringkjøbing and Thisted; under East Jutland those of Vejle, Aarhus, Randers, Viborg, Aalborg and Hjørring.

Funen and Lolland-Falster are remarkably favourable both as regards soil and shelter.

Another point which may be of importance is the question of whether hop cultivation is or is not generally carried on in the region concerned, as the cultivated plants are very apt to run wild. In districts where hop gardens are still found, there is always a chance that the species may spread and lapse into a wild state, especially if there happen to be woods near by. This may account for the fact that the wild hop is of more frequent occurrence in Funen than elsewhere, Funen being now almost the only part where hop growing is still generally carried on, the industry in Sealand, for instance, having been practically negligible for nearly a generation. Throughout the whole of Denmark, the hop gardens are gradually being given over, even in Funen, where the number of hop poles shows a marked annual decrease[1]); the hops in consequence losing their points of support, or centres of distribution. In several parts of the country, as for instance in Sealand, it is mainly in the open spaces of the forest tracts, on the edge of woods, and in wooded swamps, that the hop can find a refuge where it is able to spread in luxuriant development. The forest officials, however, inform us (*vide* p. 324—328) that the plant is regarded as a troublesome weed, which they endeavour to keep down as far as possible, and some state that the hop is therefore gradually disappearing as the forests are subjected to more rational cultivation. It is nevertheless hardly likely that the hop will ever be altogether banished, owing to the high degree of tenacity which it possesses, but in all probability the gradual decline of the hop-growing industry, together with the greater care devoted to forest culture, will in course of time render the wild hop more rare in this country than hitherto.

A question of considerable importance is whether the hop should be counted as belonging to the original flora of Denmark, or whether it was introduced by man for practical purposes cen-

[1]) By way of example, I may quote a statement made by Hr. Jørgen Rasmussen, a hop dealer of Vejrup, in Funen, who informs me that from 1900-1910, 31400 poles were taken down in the parish of Indslev alone, and in 1913 only 13000 remained in the parish. During the past three years, some 5000 more have been removed, and at the present time (1916), Indslev, formerly one of the recognised »hop districts« of Funen, counts but some 8000 hop poles in all.

turies ago, and has since spread throughout such parts of the country as offered the requisite conditions for its growth. It is doubtful whether this question will ever be solved with certainty, unless by chance remains of hops should be found in prehistoric deposits. With the known preference of the plant for wooded swamps, it would perhaps seem reasonable to suppose that the hops, for instance, might come to light in strata derived from such formation. With this idea in view, I applied, in 1912, to Dr. N. Hartz, who was then engaged on an investigation of the Danish bog deposits, and begged him to keep the matter in mind, forwarding at the same time a sample of hop seeds. In a letter dated 18th April 1912, Dr. Hartz informed me that he had never found hops in any of our prehistoric deposits, although he was very well acquainted with the appearance of the seeds themselves. It is therefore impossible to say, at any rate, for the present, whether the hop existed in this country prior to human occupation[1]).

As regards the distribution of the cultivated hop, I do not propose to go into this question in the present paper, reserving for some future occasion a discussion of the hop-growing industry in Denmark.

V. Extracts from statements made by Forest Officials as to the occurrence of the wild Hop in Danish woods and forests.

Sealand.

Skovrider Bistrup, Torbenfeldt, Mørkøv. »Hops are not tolerated in rational forest culture, which probably accounts for the fact that it is disappearing from the woods.«

Skovrider Amnitzbøll, Sorø. »The hop is found in a wild

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[1]) It is here deserving of note that V. Brøndal, in an article entitled »Humlenavne« (Danske Studier, edited by Marius Kristensen and Axel Olrik, Part 3—4, p. 185—197, Copenhagen 1915) based on philological-historical observations, concludes that hop cultivation was probably introduced into Denmark by French monks, possibly by Bishop Absalon's friend the Abbot Vilhelm of Æbeltoft, who came to Denmark in 1165. The writer states that the hop is first mentioned, albeit indirectly, by the historian Sven Aggesøn, whose work goes to the year 1185, and who mentions »*in Humla*«, the still existing *Humlum* near the Liimfjord. Direct mention is thereafter made by Henrik Harpestreng (prior to 1244) and in the Flensborg Municipal Code (1284).

state in several swamps, where it has not been purposely destroyed by the protective measures taken elsewhere.«

Skovtaxator Mourier, Næstved. »The hop appears to prefer damp, more or less swampy soil with full or nearly full light. I have most frequently found the plants growing up the stems of alder and ash, which is presumably due to the fact that the haunts of these trees in our forests for the most part coincide with the conditions of soil and moisture best suited to the hop, in addition to which, the trees in question form plantations giving sufficient access to light. The form of the stems, also, being fairly tall, not too thick, and devoid of low branches, may count for something.«

Skovrider W. Christensen, Kagerup. »The hop appears for the most part in young plantations of ash and alder in swamps and hollows, giving much troublesome work in cutting down or rooting up the plants, which would otherwise bend or break the young trees.

Skovrider H. J. Rolsted, Roskilde. »In Ryegaard Enghave wood, there are two or three small spots where the hop grows strongly under oak and pine. In Holme wood, it is found in two alder swamps, over the whole of the ground, amounting to about 1/8 acre. doing a great deal of damage to the newly planted ash saplings which are eight ft. high. I have to cut the bines away several times during the summer.«

Skovrider H. Hedemann, Lindholm, Roskilde. »The wild hop occurs in some few places in the Lindholm forest section. It twines up the ash or oak saplings in moist ground. We cut it away each year, as it would otherwise bear down the young trees, especially when under snow. When the plantations close in, the hop plants die off.«

Skovrider Thymann, Petersværft, Langebæk. »In these woods the hop soon makes its appearance wherever ash, birch and alder are planted, as also occasionally under oak.«

Skovrider Neergaard, Faxe. »Common, very troublesome weed in these woods. Found most frequently in tracts of fairly moist mould.«

Skovrider E. Ulrich, Lindersvold, Faxe. »Hops are found especially in sunny spots sheltered from the wind, as for instance a hedgerow along the southern side of the woods, and similar places; also in the copses.«

Skovrider Andersen, Giesegaard, Borup. »Wild hops are still found in the woods of the Giesegaard Stamhus estate etc. but are far less abundant now than 25 years ago, owing to the more rational method of forest culture.«

Skovrider I. F. Heger, Saltø, Næstved. »On the whole, we may doubtless say that the hop is becoming more and more rare each year, also in its wild state.«

Skovrider C. Benzon, Løvenborg, Regstrup. »In the Løvenborg woods near Løvenborg, the hop is very common in fairly moist, sandy mould, with a remarkably strong growth. It is a troublesome weed in the young plantations«.

Skovrider R. Jørgensen, Rævsvænge, Præstø. »The hop is found fairly often in a wild state on the edges of woods and in hedgerows; in a small plantation near the town of Præstø it even causes considerable trouble among the young spruce, up which it climbs.«

Skovfoged N. J. Bang, Ørnstrup, Sorø. »Found in a wild state especially in the hedgerows at the edge of the woods and in swamps, i. e. mostly in places where it does not interfere with the forest cultivation, and is therefore left undisturbed«.

Funen.

Skovrider I. Christensen, Dyrehavegaard, Kirkeby. »Wild hop is common in the woods and hedgerows. In the woods of the Lehn Barony, especially the more low-lying parts, the hop plants cause a great deal of trouble in the plantations, and have to be continually cut down all through the summer. Found frequently in peaty soil.«

Skovrider C. C. Branth, Langeskov, Stenstrup. »Wild hops found in great numbers, especially in the woods, where they do considerable damage.«

Skovrider K. Mørk Hansen, Skovlyst, Pederstrup. »The hop is common in these woods, occurring especially in low-lying parts, with fresh, mouldy soil, and is often a very troublesome weed, involving costly work in cutting it away from the young trees each year. The hops would altogether stifle their growth if left alone.«

Skovrider V. Nielsen, Holstenhus Barony, Egelund, Faaborg. »Common, especially in the richer wooded swamps.«

Skovrider J. Jørgensen, Brobyskov, Brobyværk. »There is

a great deal of wild hop in these woods; it grows especially in the wooded swamps, even where the soil is very moist.«

Skovrider V. Lassen, Wedellsborg estate, Ejby. »Fairly common in a wild state along field hedges and in the woods, where it is often troublesome in the plantations.«

Skovrider Elers Koch, Korinth. »Common in a wild state, especially in the woods and hedgerows. Disappearing, however, owing to rational culture.«

Lolland-Falster.

Skovrider Bornebusch, Hardenberg. »The hop is common in places, in the open parts of the woods, especially where the soil is moist and mouldy, or swampy. But 30—40 years ago, it grew wild in the woods to a far greater extent than now. It was then an extremely troublesome weed in many woods, whereas now, owing to the close and carefully cultivated young plantations, it is more confined to the hedges and some few moist spots.«

Skovrider Magius, Knuthenborg, Bandholm. »All through the woods and along the hedgerows, wherever the soil is moist and somewhat peaty or marshy, the hop counts among the most common growths, and is often, owing to its great luxuriance, a troublesome weed in such localities.«

Skovrider H. Marcker, Jægersbo, Rødby. »The hop is now but rarely found in a wild state in this district. It was formerly of far more frequent occurrence, causing considerable trouble especially among the osier willows; gradually, however, as these willows have partly fallen off and been replaced by other growths, the hops also have disappeared, being also far less common now in the hedgerows than formerly.«

Skovrider K. J. Lundahl, Egehus, Tingsted. »The hop is common in a wild state in the woods, especially low, moist places, and particularly among young ash and alder. Very troublesome weed in these (and other) plantations.«

Skovrider G. Wilhjelm, Orehoved. »Hops found in a wild state in several places in the woods, though not very common. Found especially in hollows with very mouldy, slightly moist (but not sour) soil, and where it does occur, generally in such abundance as to cause considerable trouble.«

Jutland.

Skovrider H. G. Oppermann, Visborggaard, Skelund. »As far as my experience goes, the wild hop requires a rich, mouldy upper soil and prefers a moist bottom. Its frequency in this district appears to be increasing, probably owing to the fact that the cattle are no longer sent to graze in the woods, as cattle prefer hops to any other forest growth.«

C. A. Mathiesen, Tapheden, Flauenskjold. »Found frequently in ash copses on the low, marshy or moist grounds in the woods of South- and East-Vendsyssel. Abundant in the wood belonging to the »Vang« farm lands in Sulsted parish, where it does a great deal of damage to the young growths of ash.«

Skovrider N. Sørensen, Felshøj pr. Hundsund. »Wild hop is common in these woods, especially in swampy ground recently planted. We cut it away each year from the young trees. Where this is not done, it grows luxuriantly, the hops produced being, in my opinion, unusually good: they are however, not turned to any account.«

VI. Summary.

The present work treats of the distribution of the wild hop in Denmark.

By sending out enquiry forms throughout the country I obtained a quantity of information, especially from forest officials, as to the occurrence of the wild hop in the various districts.

The information in question shows, that the wild hop is most common in Funen, most rare in West Jutland, the order for all divisions being as follows: 1) Funen, 2) Lolland-Falster, 3) Sealand, 4) Bornholm, 5) East Jutland, 6) West Jutland.

The hop is lacking on several small islands, as for instance a number of the islands to the south of Funen.

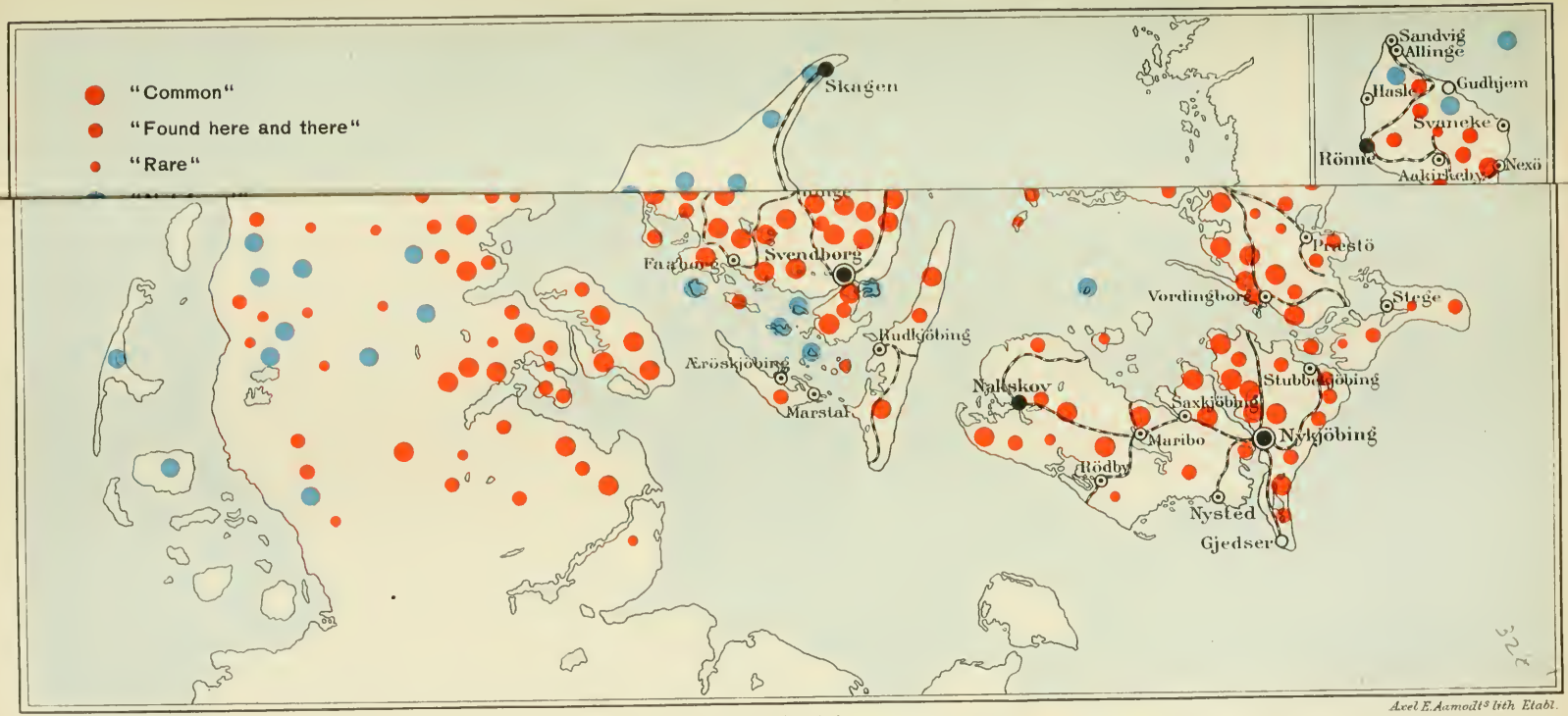
The wild hop is as a rule of little value for brewing purposes, its resins content being but poor. Examination of a number of plants from North Sealand revealed, however, some plants with up to abt. 14 % of bitter resins.

Apart from the vegetative process, the wild hop also propagates regularly by seeding. The seedlings grow slowly, and do not flower the first year, probably also but rarely in the second.

The flowering time of the wild hop has been investigated at one locality in North Sealand during the years 1911—1915;

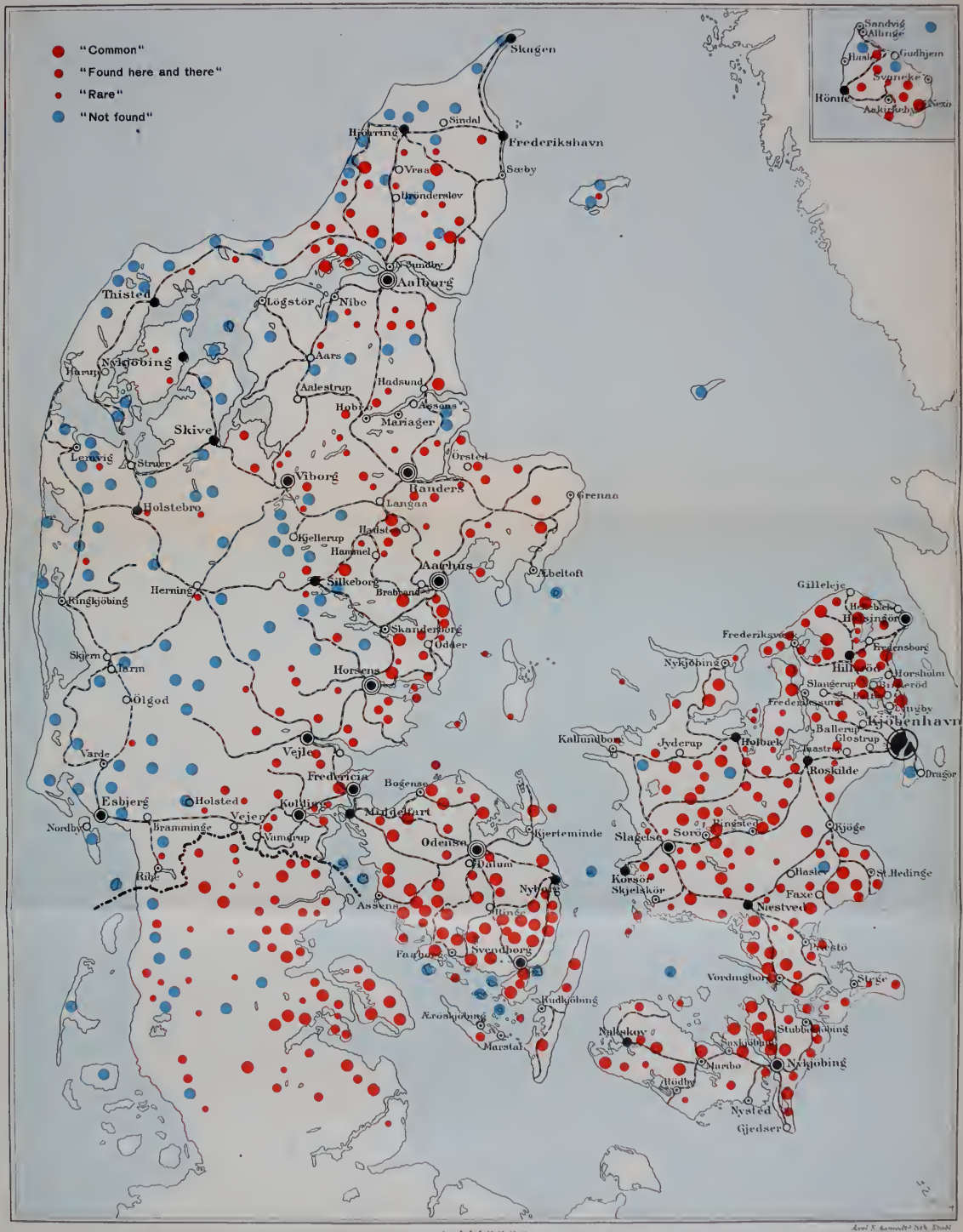
The Wild Hop (*Humulus lupulus, L.*)
Occurrence in Denmark and Northern Slesvig.

Johs. Schmidt, Comptes-Rendus des Travaux du Laboratoire de Carlsberg, vol XI.



The Wild Hop *(Humulus lupulus, L.)*
Occurrence in Denmark and Northern Slesvig.

Johs. Schmidt, Comptes-Rendus des Travaux du Laboratoire de Carlsberg, vol XI



1 : 1440000

0 10 20 30 40 50 Kilometers

0 1 2 3 4 5 6 Danish miles

Lith. af C. Schmidt's Nfl. Kbhvn.

it was found to fall early in August or late in July, the male plants commencing to flower a little earlier than the female.

It is uncertain whether the hop existed in Denmark prior to human habitation; possibly it may have been first introduced by man for practical purposes, and subsequently running wild, have spread over the country. It has not hitherto been found in prehistoric deposits in Denmark.

In the woods, the hop is regarded as a troublesome weed, and is kept down as far as possible by the forest officials. Some of these state that the hop is growing less common in our woods owing to the rational methods of forest culture.

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JOHS. SCHMIDT:

INVESTIGATIONS ON HOPS

(*Humulus lupulus*, L.).

X. ON THE AROMA IN PLANTS RAISED BY CROSSING.

BY

JOHS. SCHMIDT.

I. Introduction.

In a paper of the present series published the year before last, (V. On the Aroma of Hops, Compt. rend. du Laborat. de Carlsberg, Vol. 11, p. 149–163, 1915), I described some investigations with regard to the aroma in hops, based upon experiments carried out in the hop garden of the Laboratory.

Among the plants employed for these experiments, there was in particular a certain American hop (»Oregon Cluster«) possessing a peculiar terpentinous aroma, which is easily distinguishable, and differing widely from the aroma found in European varieties of hop[1]).

It was now shown, 1) that this »American« aroma reappeared year after year in plants cultivated in the experimental garden, and belonging to the clone received from America in 1911 (No. 60); and 2) that the »American« aroma was transmitted to abt. $^3/_4$ of the offspring derived from »Oregon 60 ♀« crossed with a Danish male plant.

Altogether, I was thus able to show, that the aroma of hops is not quite so volatile a quality as frequently supposed.

In the previous paper above mentioned, I was obliged to leave unsolved two points, the one of botanical, the other of more practical nature. To the former of these I can now return.

The question was, how far »American« aroma might be transmitted through the male parent plants to the offspring.

[1]) With regard to the American hop, vide E. S. Salmon and H. Wormald (Journal of Botany, May 1915, p. 132—135) who describe it as a species distinct from the European *Humulus lupulus*, to wit: *Humulus americanus* Nuttall.

Aroma is found, as we know, only in the female plant, being restricted to the female inflorescences or hops, and not present in the male plants. It was thus of interest to ascertain definitely whether this »American« aroma might be transmitted, not only from the mother plant to the offspring, but also from the male parent, despite the fact that this latter in itself altogether lacks the quality of »aroma«. As mentioned in the previous paper referred to (p. 161—162) crossing experiments were made with a view to deciding the point; the results of these will be dealt with in the following (II).

II. Can the Aroma of hops be transmitted through the male parent to the offspring?

The mother plant used for these experiments was a Hallertauer spät« No. 27, received several years ago from Professor F. Wagner, of Weihenstephan, in Bavaria. The male was an American plant sent me under the name of »Cluster ♂ 7 a« by Dr. W. W. Stockberger, of the Department of Agriculture, Washington, D. C., U. S. A.

The crossing was made in the summer of 1914, and the seeds sown in the spring of 1915. Unfortunately, only 3 plants came up, and of these only two flowered.

The investigations were carried out according to the manner described in the mentioned paper of 1915, and were, as then, made by the present writer and the Laboratory Assistant, mag. sc. Ö. Winge, each testing the samples independently of the other.

The result of the experiments will be seen from the following table, which has been drawn up on the same lines as the tables given in the former paper on Aroma of hops.

Plant No.	»American« Aroma (Strength 1—3[1])		Development of the hops (1—3)
	Estimated by Schmidt	Estimated by Winge	
D. 169	2	$2^1/_2$	$1^1/_2$
D. 170	1	1	$2^3/_4$

[1] 1 means slight, 2 medium and 3 strong »American« aroma.

As a result, then, it was in both cases agreed that »American« aroma was present, and we may consequently assert that a specially characteristic hop aroma can be transmitted to the offspring, even where the mother plant does not possess such aroma; i. e. the aroma must be genotypically present in the male parent plant.

III. Summary.

On crossing an American male plant (»Cluster ♂ 7 a«) with a female plant of European variety (»Hallertauer spät 27«), offspring plants were produced, the hops of which exhibited typical »American« aroma.

Despite the fact that the quality of »aroma« is altogether lacking in the male plant, being found only in the hops of the female, this quality can nevertheless be transmitted to the offspring through the male parent, in which it is genotypically present.

June 1916.

ON THE PROTEÏD SUBSTANCES OF BARLEY, IN THE GRAIN ITSELF AND DURING THE BREWING PROCESSES.

BY

H. SCHJERNING.

SECTION FOUR.
INVESTIGATIONS AS TO MALTING POWER OF VARIOUS SORTS OF BARLEY.

BY

H. SCHJERNING AND JENNY HEMPEL.

THE present work was planned by H. Schjerning in connection with his previous researches published under the same general title. The first investigations were made with certain sorts of barley grown at the experimental station at Svalöf, in Sweden, during the summer of 1912. From the autumn of 1913, the scope of the work was so extended as to include also various sorts of barley grown on Danish soil, to wit, at Tystofte experimental station, Sealand, and Abed experimental station, Lolland. From January 1914, the experimental work has been carried out by Jenny Hempel, to whom, after Schjerning's decease in November 1914, was further entrusted the task of analysing the samples harvested in the summer of 1914, and publishing the results.

Introduction.

The purpose of the present work was to ascertain whether the fact that certain sorts of barley[1]) may be easily malted out, others with difficulty, should be regarded as due to a typical hereditary quality, and if so, how far this might be affected by the nature of the soil.

[1]) Compt. rend. des travaux du Laborat. de Carlsb. **8,** 353, footnote (1910).

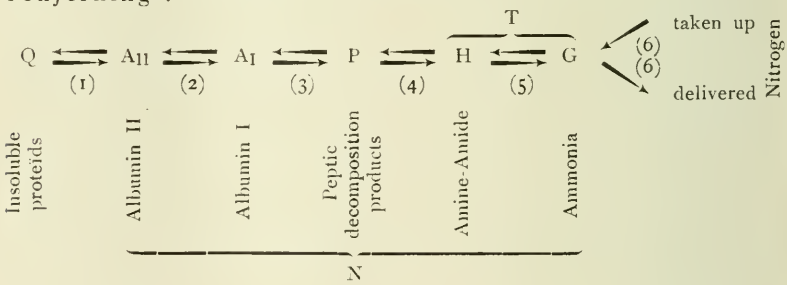
The investigations were carried out according to the methods described by H. Schjerning[1]), due regard being taken to the subsequent alterations made in these, and in the calculating processes, as set forth in his last summary review.

The nitrogenous substances have chiefly been subjected to investigation, as some of these, which may make their appearance during the malting and mashing of the barley, impair the keeping power of the beer, whereas the carbohydrates, in the methods generally adopted by Breweries, do not occasion any difficulty.

According to Schjerning, the nitrogenous substances found in barley and malt may be grouped as follows:

I Insoluble proteïds.
II Soluble matter.
1. Albumin II.
2. Albumin I.
3. Denucleïn.
4. Proteose.
5. Pepton.
6. Amine, Amide.
7. Ammonia[2]).

The ungerminated barleycorn contains chiefly insoluble proteïds, which are partially transformed, during gemination and mashing, to the mentioned substances, according to the table drawn up by Schjerning[3].



The quantity of each individual group which appears in the wort is determined by the relative velocity of the different metabolic processes (1)—(6) which have taken place during the steeping and malting of the barley, and during mashing. In a barley which shall be suited to the production of wort, i. e. a sort which "can be malted out" these metabolic processes must comply with certain conditions[4]; $(2) = (1) + a$.

[1]) Compt. rend. des travaux du Laborat. de Carlsberg **6,** 229—307 (1906); **8,** 169—401 (1910); **9,** 237—396 (1913); **11,** 45—105 (1914).
[2]) *Ibid.* **11,** 53 (1914).
[3]) *Ibid.* **11,** 54 (1914).
[4]) *Ibid.* **8,** 385 ff. (1910); **11,** 71 ff. (1914).

In other words, not only the quantity of Albumin II formed by the proteïn solution process (1), but also the amount of this group originally present, *a*, must be further transformed by the albumin conversion process (2). And in addition, it is necessary that (1) $\leqq$ (3), i. e. that the proteïn conversion process be equal to or slightly greater than the proteïn solution process. The fulfilment of the first condition here mentioned is especially important, as Albumin II sooner or later becomes precipitated in the beer, rendering it cloudy and less able to keep[1]). This is not the case, however, with Albumin I, or the other groups of soluble matter.

Besides the nitrogenous compounds, the quantity of wort extract, which may best be taken as a measure of the amount of carbohydrates dissolved[2]), has also been determined; it should, according to Schjerning[3]), make up at least 73 % of the dry matter in the malt. The quantity of acid in the wort, and the concentration of hydrogen ions is determined, as also the intensity of the oxidation processes and root formation; this last should give at least 3 g. of rootlets pr. 100 g. malt grains[4]). The investigations have included extract from the steeped, ungerminated corn, the steep, and wort produced from malt, as both series of determinations are required in order to show the course of the separate processes.

The grain used in the experiments included some samples from Svalöf experimental station, grown in three successive years, 1912—14 (Section I), and others from the experimental stations at Tystofte and Abed (Section II); the last-named samples were from the years 1913—1914.

As will be seen from the following detailed record of the results arrived at, the experiments have not furnished any answer to the question at issue; whether the degree of "maltability" is or is not dependent upon the sort of grain. It was indeed hardly to be expected that experiments extending over so short a period of years should here suffice, as the problems involved are of a highly complicated nature, and are subject, moreover, to the influence of external factors, — nature of soil, weather conditions — which are only in a slight degree amenable to control.

The results obtained may be briefly summarised as follows:

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[1]) Compt. rend. des travaux du Laborat. de Carlsberg **11,** 71 (1914).
[2]) *Ibid.* **8,** 197 ff. (1910).
[3]) *Ibid.* **8,** 387 (1910).
[4]) *Ibid.* **8,** 387 (1910).

The samples examined exhibited no typical racial peculiarity in regard to malting power; in no case was the relative velocity of the different metabolic processes of such a character as constantly to bring about the disappearance of Albumin II after a suitable malting time. It would seem, however, that one race (Golden barley) is, in comparison with the others, particularly poor in proteolytic enzymes, the conversion of indissoluble proteïds in the corn being here relatively slight.

Again, as regards the conversion of insoluble carbohydrates — here measured by formation of extract — no typical racial peculiarity was discernible in the samples investigated; the formation of extract is more or less uniform in all cases, attaining or exceeding the requisite 73 % of dry malt-stuff.

Similarly, with regard to acid formation, loss by oxidation, and root growth, no typical racial difference could be observed. Possibly some such may be shown to exist in the weight of the corn for several samples; this is, however, of no interest in connection with the suitability of the grain for malting purposes.

Experimental methods and Calculation.

600 g. air-dried corn of each sort was steeped in distilled water. The duration of the steeping period[1]) was determined for all the various sorts for each year and from each locality, and is shown in Tables I, XI and XII.

200 g. of the normally steeped grain was dried for 24 hours in a kiln[2]); all kiln-dried material was kept in glass-stoppered bottles until required for examination.

The steep intended for chemical analysis was prepared in the following manner: it was ground to a fine powder, and the percentage of dry matter, *b*, determined by drying a sample of abt. 10 g. at 100—105° for 24 hours. 50 g. of the powder was used for analysis. This was mixed with 200 cc. distilled water, at ordinary indoor temperature, and worked up to an even consistency, then left to stand for an hour, with occasional stirring. Water was now added until the total weight of the mixture reached 450 g. The whole was then filtered through a dry filter-paper, the first cloudy filtrate being poured through the filter

[1]) Compt. rend. des travaux du Laborat. de Carlsberg **8,** 284 ff. (1910).
[2]) *Ibid.* **8,** 181 ff. (1910).

again. The specific gravity of the filtrate was ascertained, and the quantity of extract in the liquid thence calculated according to Balling's Tables; more water was then added, until 100 cc. of this "dilute wort" answered to 10 g. kiln-dried steep[1]). The wort was shaken up with toluol, filtered, and stored in an ice-chest.

The remainder of the steep was laid out to germinate at 14—16°[2]) in a germinating apparatus[3]), through which was passed a current of water-saturated air at abt. 10 l. per hour. Where the corns became too dry during the germinating process, a suitable quantity of water was added; as a rule this was not required. After the third day, the malt was daily loosened from the metal grid of the apparatus and "thrown" so as to avoid damaging the rootlets. Germination was interrupted, in the case of the two-rowed sorts, as a rule after the lapse of 8, 10 and 13 days, and samples of abt. 220 g. taken and kiln-dried as with the steep. Rootlets and any leaf shoots (hussars) were removed from the grain[4]), weighed in a kiln-dried state and after drying at a temperature of 100— 105° for 24 hours; from this the percentage of dry matter, s, in the kiln-dried rootlets was calculated. The weight, q, of the total kiln-dried malt grains was determined together with the weight, a, of 1000 grains.

The malt grains were ground to a fine powder, and the percentage of dry matter, b, determined as before. 50 g. of the powder was used for mashing[5]), and the "dilute wort" thus produced, 100 g. of which answered to 10 g. kiln-dried malt, used for analyses. The nitrogenous groups above mentioned were determined according to Schjerning's methods[6]), and formol-titratable nitrogen by that of S. P. L. Sørensen[7]) after previous precipitation of weak acids with barium hydroxide, barium chloride having first been added[8]). The quantity of acid was determined by titration with n/10 sodium hydroxide, using phenolphtaleïn as indicator[9]). The determinations as to concentration of hydrogen

[1]) Compt. rend. des travaux du Laborat. de Carlsberg **8,** 191 (1910).
[2]) *Ibid.* **8,** 230 ff. (1910).
[3]) *Ibid.* **8,** 179 ff. (1910).
[4]) *Ibid.* **8,** 189 1910).
[5]) *Ibid.* **8,** 189 ff. (1910).
[6]) *Ibid.* **11,** 46 ff. (1910).
[7]) *Ibid.* **7,** 1 ff. (1907).
[8]) *Ibid.* **9,** 239 footnote 4 (1913).
[9]) *Ibid.* **9,** 239 (1913).

ions were carried out according to S. P. L. Sørensen's colorimetric method[1]); in some cases also by the electrometric process[2]).

Tables II, V, VIII, XIII, XVI, XIX, XXII, XXV show the direct analysis figures. In Tables III, VI, IX, XIV, XVII, XX, XXIII, XXVI, the values found have been worked out to apply for 10000 grains[3]). Precisely as in Schjerning's last paper, of 1914, the extract percentage of malt dry matter is here designated by Ex, while Gx, G, Q and N indicate respectively the quantity of extract, of ammonia-nitrate, of nitrogen in the insoluble and nitrogen in soluble compounds for 10000 grains[4]). Finally, R' denotes the weight of rootlets corresponding to 100 g. malt grains calculated according to the formula 100 q : p, where p is the weight of rootlets and q that of the corresponding malt grains.

Calculation of the numerical values for the metabolic processes (1)—(6) was carried out according to the method given in Schjerning's last paper[5]).

In Tables IV, VII, X, XV, XVIII, XXI, XXIV, XXVII some of the results have been worked out to correspond to 1000 g. dry barley matter, as a means of measuring the amount obtained from the various sorts.

Experimental section.

Section I. Barley from Svalöf Experimental Station.

From Svalöf Experimental Station we have, for the three successive years 1912, 1913 and 1914, six different sorts of barley for investigation: Hannchen, Chevallier II, Swan's neck, Primus, Princess, and Golden barley. In 1912 and 1914, the samples were grown at Svalöf itself; in 1913 at Limhamn.

Table I shows the results of the steeping experiments, from which it will be seen, 1) that the normal steeping time for the different sorts differs for the three years, being generally somewhat higher for 1913 than for 1912, and for 1914 somewhat higher again. The external factors of weather and soil must have affected the results here, the difference between 1912 and 1914

[1]) Compt. rend. des travaux du Laborat. de Carlsberg **8,** 67 ff. (1910).
[2]) *Ibid.* **8,** 20 ff. (1910).
[3]) *Ibid.* **8,** 191 (1910).
[4]) *Ibid.* **11,** 53 (1914).
[5]) *Ibid.* **11,** 55 (1914).

being presumably due more especially to the weather conditions, since the samples for these two years were both grown at the same place. 2) Primus and Golden barley would seem to be characterised by a longer normal steeping time than the four other sorts examined, which differ little in this respect one from another. As regards oversteeping, Hannchen is far more susceptible to this than are the remaining sorts.

Tables II, V og VIII show that racial differences are discernible, *inter alia,* from the weight of the grain; Golden barley is on the whole that showing the smallest grain of those here concerned, while Princess, and particularly Primus, are comparatively large-grained. This is hardly of interest as regards the brewing industry, but is worthy of note from a purely botanical point of view, affording as it does a parallel to the fact noted by W. Johannsen[1]) in the case of beans; that the size of the seed is a character mark of hereditary value. This will also be noticed under consideration of the barley grown on Danish soil (p. 354).

The formation of extract has in practically all cases attained the same value in wort prepared from 8 days' malt as in that from 10 and 13 days' malt, (*vide* figures in column Ex, Tables III, VI and IX). The formation of enzymes splitting carbohydrates would thus have reached its maximum after the lapse of only 8 days. When worked out for 10000 grains (*vide* column Gx) the values for quantity of extract appeared in almost all cases slightly higher in wort prepared from 8 days' malt than in that from 10 and 13 days' malt; this is due to the increasing loss of matter by root growth, possible growth of leaf-shoots, and by the respiratory process. Calculated for 1000 g. dry barley matter, (*vide* Gx_1 in Tables IV, VII and X) the yield of extract varies between 765 and 596 gr. but these figures do not indicate any difference in the yielding capacity of the various sorts.

The quantity of rootlet, again, shows no marked racial difference; possibly the growth velocity in Swan's neck and Princess is a little higher than in the remaining sorts (*vide* figures under R i Tables III, VI and IX, and R_1 in Tables IV, VII and X).

The values for concentration of hydrogen ions all lie within the limits of $10^{\div 5.5}$ and $10^{\div 6.1}$; there is no dependent relation

[1]) W. Johannsen: Elemente der exakten Erblichkeitslehre (1913). p. 158 ff.

TABLE I. **Barley from Sweden.**

Harvest		1912											
Locality		Svalöf											
Soil		Medium stiff clay											
Weather		Very rainy and unfavourable											
Sort		Hannchen		Chevallier II		Swan's neck		Primus		Princess		Golden barley	
% of germinative		energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity
Steeped for ... hours in distilled water at 18—20°	6	97.2	99.4	98.0	99.8	97.8	99.6	97.0	99.6	97.4	99.6	93.2	99.2
	24	96.6	98.8	96.6	99.2	98.4	99.0	97.6	99.2	99.6	100.0	96.6	99.4
	48	91.8	95.2	95.2	99.4	98.0	99.8	99.0	99.4	97.6	99.8	98.0	99.4
	72	89.2	95.2	93.2	97.6	96.8	99.2	94.6	98.8	96.8	99.0	96.2	98.4
	96	46.4	83.0	79.8	93.8	88.8	95.6	89.0	99.2	96.0	99.4	96.0	98.4

Harvest		1913											
Locality		Limhamn											
Soil		Light, chalky, first class soil											
Weather		pretty favourable											
Sort		Hannchen		Chevallier II		Swan's neck		Primus		Princess		Golden barley	
% of germinative		energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity
Steeped for ... hours in distilled water at 18—20°	6	87.2	99.8	84.4	99.4	95.8	98.8	89.4	99.4	93.4	98.8	98.4	99.4
	24	98.8	99.6	98.2	99.0	98.2	99.4	96.4	98.4	97.0	98.4	99.0	99.0
	48	94.4	98.4	95.6	99.2	97.0	99.6	97.0	98.2	96.2	99.4	98.8	99.2
	72	76.0	95.2	96.0	98.0	94.2	97.4	95.0	98.8	93.2	97.4	98.4	99.2
	96	24.6	86.8	89.6	94.6	91.6	96.6	92.4	98.4	92.4	97.0	99.0	99.0

Harvest		1914											
Locality		Svalöf											
Soil		Medium stiff clay											
Weather		favourable											
Sort		Hannchen		Chevallier II		Swan's neck		Primus		Princess		Golden barley	
% of germinative		energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity
Steeped for ... hours in distilled water at 18—20°	6	99.6	100.0	88.4	100.0	98.6	99.6	95.0	99.2	99.8	100	99.6	99.6
	24	100.0	100.0	99.6	99.6	98.6	99.0	99.0	99.2	99.8	99.8	99 4	99.4
	48	98.8	99.0	99.8	100.0	98.6	99.0	99.0	99.4	99.6	99.6	99.2	99.6
	72	47.0	97.0	100.0	100.0	98.4	99.2	98.8	99.0	98.4	98.8	99.2	99.8
	96	7.4	87.0	89.0	97.0	95.6	98.6	98.6	99.2	98.8	99.6	99.2	99.6

Permissible error = 2 %.

TABLE II. Year 1912. Barley from Svalöf.

Found for value		a	b	m	c	p	s	q	k	U	h	e	f							g	pH	10000 barley corns contain g. dry matter	% dry matter in the barley corns	Normal steeping time of the sample in hours
Sort	After conclusion of normal steeping germinated at 14—16° in . . . days [1]	1000 wet corns weigh g.	% dry matter in the corns	g. hydrous matter	Containing nitrogen ∽ cc n/10 acid	Quantity of rootlet: Root: g. rootlets	Quantity of rootlet: Root: with % dry matter	Quantity of rootlet: Corn: g. Malt grains	% Extract (Ball.)	25 cc. dilute wort ∞ no of corns	Acid ∞ cc n/10 NaOH	25 cc. dilute wort contains: Nitrogen ∞ cc n/10 Acid as: Total nitrogen	precipitable by: $SnCl_2$	$HgCl_2$	$FeAc_3$	$UrAc_2$	$MgSO_4$	formol-titratable	50 cc. dilute wort contains nitrogen in form of ammonia ∞ cc n/10 acid				
Hannchen	Steep	0	43.51	91.92	2.7687	31.2	—	—	—	1.30	57.5	—	3.8	1.5	2.2	2.2	2.9	1.8	1.5	0.1	—			
		8	39.56	91.67	5.0754	54.0	6.0791	91.93	132.6	8.48	63.2	2.5	11.2	1.4	3.6	3.7	5.0	2.8	4.0	0.5	6.1	407.7	87.73	24
	Malt	10	38.01	92.86	3.3370	36.8	7.1198	93.38	130.1	8.55	65.5	2.6	11.6	1.4	3.7	3.8	5.3	2.9	4.6	—	6.1			
		13	38.41	92.85	2.8305	31.6	8.6620	93.86	138.1	8.48	65.1	2.6	12.0	1.5	3.7	3.8	5.4	2.9	4.5	0.6	6.1			
Chevall. II	Steep	0	44.70	91.05	3.1658	35.2	—	—	—	0.88	55.9	1.7	3.3	1.3	1.9	1.9	2.4	1.5	1.6	0.1	5.9			
		8	40.59	91.10	4.0022	42.4	6.4887	91.93	131.1	8.24	61.6	2.6	10.3	1.2	3.2	3.4	5.0	2.7	4.0	0.3	6.1	414.6	89.33	24
	Malt	10	39.26	92.25	4.1105	44.4	7.7522	93.49	140.2	8.31	63.7	2.6	10.7	1.4	3.2	3.7	5.1	2.7	3.9	0.3	6.1			
		13	39.09	92.20	4.5255	49.2	8.1991	93.69	131.0	8.29	64.0	2.7	10.7	1.3	3.3	3.7	5.3	2.7	4.0	0.3	6.1			
Swan's neck	Steep	0	43.12	93.55	3.0017	38.4	—	—	—	0.91	58.0	1.6	3.9	1.5	2.3	2.3	3.0	1.9	1.9	0.2	6.0			
		8	38.09	92.23	4.2925	52.8	7.7901	92.11	108.3	8.30	65.6	3.2	14.3	1.0	3.7	4 4	6.5	3.0	5.9	0.5	6.0	409.1	88.43	48
	Malt	10	35.84	92.84	4.3797	52.8	12.0416	93.91	110.2	8.34	69.8	3.5	15.1	1.3	3.7	4.6	6.8	2.7	6.7	—	6.0			
		13	34.68	89.87	3.3523	42.0	13.2647	91.92	112.2	7.98	72.1	4.0	16.1	1.0	3.6	4.5	7.1	2.4	7.5	0.8	6.0			
Primus	Steep	0	45.92	92.74	3.2662	38.8	—	—	—	0.86	54.4	1.6	3.6	1.6	2.1	2.1	2.7	1.6	2.1	0.1	5.9			
		8	43.06	89.50	3.2672	36.0	7.9397	91.98	141.9	8 06	58.1	2.6	11.2	1.5	3.6	4.0	5.8	3.0	4.3	0.4	5.9	432.8	88.62	48
	Malt	10	40.52	92.65	4.5840	52.8	8.9790	93.73	136.7	8.31	61.7	2.8	11.9	1.6	3.6	4.2	6.2	3.2	4.9	0.4	5.9			
		13	40.51	89.20	3.2615	36.8	11.1200	90.22	137.0	7.98	61.7	3.0	12.1	1.7	3.6	4.3	6.3	3.2	4.8	0.4	5.9			
Princess	Steep	0	48.24	93.20	2.4478	27.6	—	—	—	0.77	51.8	1.4	3.4	1.5	2.0	2.0	2.7	1.6	1.9	0.1	6.1			
		8	43.95	92.04	3.5070	36.0	7.7043	92.21	114.3	8.34	56.9	2.5	10.9	1.3	3.5	3.6	5.2	2.5	4.9	0.6	6.1	459.2	88.95	48
	Malt	10	39.49	92.99	3.6536	36.0	11.5161	93.37	98.1	8.51	63.3	2.9	11.8	1.2	3.2	3.8	5.3	2.3	5.2	0.5	6.1			
		13	38.63	91.27	3.2025	32.4	12.8945	91.86	103.5	8.30	64.7	3.0	11.3	1.2	3.1	3.7	5.5	2.2	5.2	0.5	6.1			
Gold. barley	Steep	0	40.90	92.15	2.9053	33.6	—	—	—	0.81	61.1	1.5	3.3	1.2	1.8	1.8	2.5	1.5	2.4	0.1	6.0			
		8	37.34	90.83	3.4455	37.6	8.2285	91.36	145.7	8.14	67.0	2.7	10.8	1.0	3.4	3.5	5.3	2.7	4.9	0.4	6.1	389.4	89.25	48
	Malt	10	35.93	92.77	3.6810	41.6	7.9220	92.62	118.5	8.34	69.6	2.8	11.2	1.0	3.4	3.8	5.6	2.8	5.2	0.4	6.1			
		13	35.31	90.63	3.1212	34.8	10.0840	92.32	133.1	8.16	70.8	2.9	11.7	1.0	3.6	4.1	6.0	2.7	5.5	0.4	6.1			

[1] = days of 24 hours.

TABLE III. **Year 191**

Columns marked			A	Y	Gx	R	I	S	A_I	A_{II}	D	E	F	G	H	O
			10 000 corns gave													
Sort		After conclusion of normal steeping, germinated at 14—16° in ... days	g. dry matter	g. unaffected matter (husk)	g. Extract	g. loss as rootlets	g. loss by oxidation	Acid ∞ cc. n. NaOH	g. nitrogen in the form: Albumin I	Albumin II	peptic decomposition products = P: Denucleïn	Propepton	Pepton	tryptic decomposition products = T: Ammonia	Amine-Amide	peptic-bound nitrogen
Hannchen	Steep	0	399.9	363.3	36.6	0	0		0.37	0.07	0.10	0.00	0.17	0.01	0.21	0.37
	Malt	8	362.7	66.4	296.3	16.7	20.5	39.6	0.31	0.29	0.20	0.02	0.29	0.05	1.32	0.89
		10	353.0	66.1	286.9	19.4	27.5	39.7	0.30	0.30	0.19	0.02	0.32	1.35		0.98
		13	356.6	69.4	287.2	22.7	20.6	39.9	0.32	0.28	0.20	0.02	0.34	0.06	1.36	0.97
Chevallier II	Steep	0	407.0	374.9	32.1	0	0	30.4	0.33	0.05	0.10	0.00	0.12	0.01	0.22	0.40
	Malt	8	369.8	74.9	294.9	18.5	18.7	42.2	0.27	0.30	0.16	0.04	0.37	0.03	1.17	0.91
		10	362.2	74.5	287.7	20.3	24.5	40.8	0.31	0.17	0.22	0.11	0.31	0.03	1.20	0.86
		13	360.4	75.0	285.4	22.9	23.7	42.2	0.28	0.22	0.22	0.09	0.35	0.03	1.15	0.88
Swan's neck	Steep	0	403.4	371.5	31.9	0	0	27.6	0.36	0.10	0.10	0.00	0.16	0.02	0.20	0.46
	Malt	8	351.3	72.8	278.5	25.2[1]	26.9[1]	48.8	0.21	0.28	0.30	0.15	0.45	0.05	1.61	1.26
		10	332.7	69.5	263.2	36.5[1]	34.2[1]	50.1	0.26	0.10	0.38	0.18	0.44	1.67		1.34
		13	311.7	68.0	243.7	37.7[1]	54.0[1]	55.4	0.19	0.11	0.40	0.17	0.51	0.08	1.67	1.46
Primus	Steep	0	425.9	393.8	32.1	0	0	29.4	0.41	0.00	0.13	0.00	0.15	0.01	0.23	0.54
	Malt	8	385.4	79.5	305.9	22.2	18.3	44.8	0.36	0.27	0.24	0.09	0.44	0.04	1.26	1.04
		10	375.4	78.9	296.5	24.9	25.6	45.4	0.36	0.24	0.22	0.13	0.46	0.04	1.25	1.11
		13	361.3	76.5	284.8	29.7	34.9	48.6	0.39	0.18	0.25	0.16	0.45	0.04	1.28	1.09
Princess	Steep	0	449.6	419.4	30.2	0	0	27.0	0.41	0.02	0.11	0.00	0.19	0.01	0.18	0.51
	Malt	8	404.5	81.4	323.1	27.3[1]	17.8[1]	43.9	0.32	0.27	0.27	0.03	0.39	0.06	1.34	1.21
		10	367.2	70.8	296.4	43.3[1]	39.1[1]	45.8	0.27	0.11	0.33	0.13	0.33	0.05	1.39	1.15
		13	352.6	69.8	282.8	44.2[1]	52.8[1]	46.4	0.26	0.09	0.32	0.13	0.39	0.05	1.21	1.13
Golden barley	Steep	0	376.9	349.9	27.0	0	0	24.5	0.28	0.06	0.07	0.00	0.16	0.01	0.18	0.55
	Malt	8	339.2	71.4	267.8	19.3	18.4	40.3	0.21	0.33	0.17	0.02	0.38	0.04	1.11	1.02
		10	333.3	69.5	263.8	22.3	21.3	40.2	0.20	0.28	0.20	0.08	0.37	0.04	1.08	1.09
		13	320.0	66.1	253.9	24.7	32.0	40.9	0.20	0.23	0.28	0.10	0.38	0.04	1.08	1.06
Analytical error			2.1		2.6	2.4		3.3	0.07							

[1] As these samples contained many hussars, the determination of rootlet wastage will be somewhat too high, and

rley from Svalöf.

n	Q = n÷N	N	P	T	P+T		α	Ds	Cv	Pa	Ta	Aml	Rn	Ps	Ex	R′	
								Numerical value of . . . process									
								(1	(2	(3	(4	(5)	(6)				
Total of nitrogen	insoluble compounds	soluble decomposition products	peptic	tryptic	proteolytic	pH.	10,000 steeped corns contain g. nitrogen in form of albumin II	Proteidsolution:	Albumin conversion	Peptic action	Tryptic action	Ammonia liberation	Root formation	Peptic action	% Extract of dry malt stuff	g. rootlets for 100 g. malt grains	hussars
.86	5.93	0.93	0.27	0.22	0.49		0.07	—	—	—	—	—	—	—	9.15	0	
.89	3.41	2.48	0.51	1.37	1.88	6.1		2.52	2.30	2.36	2.12	1.01	0.97	0.52	81.70	4.6	
.87	3.39	2.48	0.53	1.35	1.88	6.1		2.54	2.31	2.38	2.12		0.99	0.61	81.26	5.5	some few
.00	3.42	2.58	0.56	1.42	1.98	6.1		2.51	2.30	2.35	2.06	0.91	0.86	0.60	80.54	6.3	several
.96	6.13	0.83	0.22	0.23	0.45	5.9	0.05	—	—	—	—	—	—	—	7.89	0	
.02	3.68	2.34	0.57	1.20	1.77	6.1		2.45	2.20	2.26	1.91	0.96	0.94	0.51	79.74	4.9	
.94	3.59	2.35	0.64	1.23	1.87	6.1		2.54	2.42	2.44	2.02	1.04	1.02	0.46	79.42	5.5	some few
.95	3.61	2.34	0.66	1.18	1.84	6.1		2.52	2.35	2.40	1.96	1.03	1.01	0.48	79.20	6.3	several
.72	6.78	0.94	0.26	0 22	0.48	6.0	0.10	—	—	—	—	—	—	—	7.92	0	
.56	3.51	3.05	0.90	1.66	2.56	6.0		3 27	3.09	3.24	2.60	1.19	1.16	0.80	79.27	7.2[1]	a good few
.05	3.02	3.03	1.00	1.67	2.67	6.0		3.76	3.76	3.86	3.12		1.67	0.88	79.10	10.9[1]	extremely numerous
.08	2.95	3.13	1.08	1.75	2.83	6.0		3.83	3.82	3.90	3.17	1.70	1.64	1.00	78.17	11.8[1]	extraordinarily numerous
.64	6.71	0.93	0.28	0.24	0.52	5.9	0.00	—	—	—	—	—	—	—	7.54	0	
.64	3.94	2.70	0.77	1.30	2.07	5.9		2.77	2.50	2.55	2.16	1.03	1.00	0.50	79.38	5.6	
.53	3.83	2.70	0.81	1.29	2.10	5.9		2.88	2.64	2.69	2.20	1.14	1.11	0.57	78.98	6.6	some few
.39	3.64	2.75	0.86	1.32	2.18	5.9		3.07	2.89	2.91	2.37	1.28	1.25	0.55	78.82	8.1	some few
.61	6.69	0.92	0.30	0.19	0.49	6.1	0.02	—	—	—	—	—	—	—	6.71	0	
.32	3.64	2.68	0.69	1.40	2.09	6.1		3.05	2.80	2.89	2.50	1.34	1.29	0.70	79.88	6.7[1]	many
.45	2.84	2.61	0.79	1.44	2.23	6.1		3.85	3.76	3.90	3.41	2.20	2.16	0.64	80.72	11.7[1]	extremely numerous
.47	3.02	2.45	0.84	1.26	2.10	6.1		3.67	3.60	3.75	3.21	2.18	2.14	0.62	80.20	12.4[1]	extraordinarily numerous
.62	5.86	0.76	0.23	0.19	0.42	6.0	0.06	—	—	—	—	—	—	—	7.16	0	
.70	3.44	2.26	0.57	1.15	1.72	6.1		2.42	2.15	2.22	1.88	0.95	0.92	0.47	78.94	5.6	some few
.68	3.43	2.25	0.65	1.12	1.77	6.1		2.43	2.21	2.29	1.87	0.97	0.94	0.50	79.16	6.7	some few
.51	3.20	2.31	0.76	1.12	1.88	6.1		2.66	2.49	2.57	2.04	1.14	1.11	0.54	79.34	7.6	several
.13	0.12	0.05		0.07		0.1					0.07						

ss by oxidation correspondingly too low.

TABLE IV. Year 1912. Barley from Svalöf. For 1000 g. dry matter.

Columns marked			R_1	Gx_1	S_1	n_1	N_1	AI_1	AII_1	P_1	T_1	P_1+T_1	O_1
Sort		After conclusion of normal steeping, germinat. at 14-16° in — days	g. rootlets	g. extract	Acid ∾ cc.³ n. NaOH	g. Nitrogen in form of: Total of nitrogen	soluble compounds	Albumin: I	Albumin: II	decomposition products: peptic	decomposition products: tryptic	decomposition products: proteolytic	peptide-bound nitrogen
Hannchen	Steep	0	—	89.8	—	16.83	2.28	0.91	0.17	0.66	0.54	1.20	0.91
	Malt	8	41.0	726.8	97.1	14.45	6.08	0.76	0.71	1.25	3.36	4.61	2.18
		10	47.6	703.8	97.4	14.40	6.08	0.74	0.74	1.30	3.31	4.61	2.40
		13	55.7	704.5	97.9	14.72	6.33	0.78	0.69	1.37	3.48	4.85	2.38
Chevallier II	Steep	0	—	77.4	73.3	16.79	2.00	0.80	0.12	0.53	0.55	1.08	0.96
	Malt	8	44.6	711.3	101.8	14.52	5.64	0.65	0.72	1.37	2.89	4.26	2.19
		10	49.0	693.9	98.4	14.33	5.67	0.75	0.41	1.54	2.97	4.51	2.07
		13	55.2	688.4	101.8	14.35	5.64	0.68	0.53	1.59	2.85	4.44	2.12
Swan's neck	Steep	0	—	78.0	67.5	18.87	2.30	0.88	0.24	0.64	0.54	1.18	1.12
	Malt	8	61.6	680.7	119.3	16.03	7.45	0.51	0.68	2.20	4.06	6.26	3.08
		10	89.2	643 3	122.4	14 79	7.41	0.64	0.24	2.44	4.08	6,52	3.27
		13	92.1	595.6	135.4	14.86	7.65	0.46	0.27	2.64	4.28	6.92	3.57
Primus	Steep	0	—	74.2	67.9	17.66	2.15	0.95	0.00	0.65	0.55	1.20	1.25
	Malt	8	51.3	706.9	103.5	15.35	6.24	0.83	0.62	1.78	3.00	4.78	2.40
		10	57.5	685.2	104.9	15.09	6.24	0.83	0.55	1.87	2.98	4.85	2.57
		13	68.6	658.2	112.3	14.77	6.36	0.90	0.42	1.99	3.05	5.04	2.52
Princess	Steep	0	—	65.8	58.8	16.57	2.00	0.89	0.04	0.65	0.41	1.06	1.11
	Malt	8	59.5	703.7	95.6	13.76	5.84	0.70	0.59	1.50	3.05	4.55	2.64
		10	94.3	645.6	99.8	11.87	5.68	0.59	0.24	1.72	3.14	4.86	2.50
		13	96.3	615.9	101.1	11.91	5.34	0.57	0.20	1.83	2.74	4.57	2.46
Golden barley	Steep	0	—	69.3	62.9	17.00	1.95	0.72	0.15	0.59	0.49	1.08	1.41
	Malt	8	49.6	687.7	103.5	14.64	5.80	0.54	0.85	1.46	2.95	4.41	2.62
		10	57.3	677.4	103.2	14 59	5.78	0.51	0.72	1.67	2.88	4.55	2.70
		13	63.4	652.0	105.0	14.15	5.93	0.51	0.59	1.95	2.88	4.83	2.80
Analytical error c.			6.4	6.9	8.7	0.34	0.13				0.19		

Found for value		Sort	a	b	m	c	p	s	q	k	U	h	e	f					g			
							Quantity of rootlet						25 cc. dilute wort contains									
			1000 wet corns weigh gr.	% dry matter in the corns	gr. hydrous matter	Containing nitrogen ∽ cc. ^{3} n/$_{10}$ acid	gr. rootlets	Root with % dry matter	Corn gr. malt grains	% extract (Ball.)	∽ no. of corns 25 cc. dilute wort	acid ∽ n/$_{10}$ NaOH	Total nitrogen	precipitable by				Acid as formol-titrable	50 cc. dilute wort contains nitrogen in form of ammonia ∽ cc. n/$_{10}$ acid	pH	1000 barley corns contain gr. dry matter	% dry matter in the barley corns	Normal sleeping time of the sample in hours
														$SnCl_2$	$HgCl_2$	$FeAc_3$	$PbAc_2$	$MgSO_4$				
After conclusion of normal sleeping, germinated at 14—16° in . . . days[1])													Nitrogen ∽ cc. n/$_{10}$ Acid as									
	Steep	Hannchen	43.07	92.25	4.3187	40.7	—	—	—	0.78	58.0	0.9	2.6	0.9	1.5	1.6	1.7	1.3	0.5	0.1	5.9	
	8		39.53	93.08	4.0027	36.9	5.4301	93.69	136.6	8.61	63.3	1.8	10.3	1.3	3.0	3.2	3.8	2.4	3.0	0.3	6.0	
	Malt 10		39.20	92.02	3.9777	36.5	5.8665	92.73	131.3	8.66	63.8	1.9	10.8	0.7	3.0	3.3	4.0	2.3	3.0	0.3	6.0	409.1
	13		39.25	90.26	4.1555	39.0	6.4992	93.94	136.8	8.49	63.7	2.0	10.9	0.8	3.1	3.4	4.2	2.4	3.0	0.3	6.0	
	Steep	Chevall. II	45.37	90.17	4.5434	42.5	—	—	—	0.65	55.1	0.9	2.4	0.6	1.3	1.4	1.6	1.2	0.8	0.1	5.9	
	8		40.78	93.51	3.6765	33.0	5.6202	94.04	128.4	8.44	61.3	1.9	10.4	0.8	2.7	3.0	3.9	2.0	3.5	0.3	5.9	
	Malt 10		39.98	92.77	3.1590	28.5	6.3071	93.28	122.9	8.37	62.5	2.0	10.0	0.6	2.7	3.0	3.9	1.9	3.5	(0.2)	5.9	421.0
	13		39.41	91.23	3.1055	29.0	8.6105	93.92	144.9	8.24	63.4	2.0	10.8	0.6	2.8	3.2	3.9	2.0	3.4	(0.3)	5.9	
	Steep	Swan's neck	48.63	91.17	4.4055	47.5	—	—	—	0.81	51.4	1.1	2.5	0.8	1.4	1.5	1.8	1.2	0.6	—	5.8	
	8		44.83	90.03	4.0338	42.5	5.9183	90.37	130.9	8.24	55.8	2.4	12.9	1.0	3.4	3.6	4.7	2.5	4.5	0.4	5.9	
	Malt 10		43.45	91.75	4.1145	43.0	6.9222	91.78	126.1	8.41	57.5	2.6	13.3	0.9	3.3	3.7	5.1	2.5	4.5	—	5.9	446.9
	13		42.33	92.40	3.9545	42.5	8.2176	93.24	142.0	8.49	59.1	2.8	14.7	1.0	3.6	4.4	5.2	2.6	4.9	0.4	5.9	
	Steep	Primus	51.41	91.20	3.8990	37.2	—	—	—	0.78	48.6	1.1	2.4	0.8	1.2	1.2	1.4	0.8	0.5	0.1	5.8	
	8		49.93	89.26	3.9406	35.7	4.5956	90.28	137.6	8.27	50.1	2.0	9.9	1.2	3.2	3.2	4.1	2.5	3.1	0.3	5.9	
	Malt 10		47.89	91.32	4.0922	38.1	5.3205	91.23	132.2	8.39	52.2	2.1	10.4	1.1	3.3	3.3	4.5	2.6	3.0	0.3	5.9	476.4
	13		45.85	91.49	4.0638	38.2	6.5289	93.02	147.9	8.58	54.5	2.2	11.3	1.2	3.3	3.6	4.5	2.7	3.2	—	5.9	
	Steep	Princess	44.57	90.92	4.1942	44.5	—	—	—	0.70	56.1	0.8	2.4	0.8	1.2	1.4	—	1.1	0.7	—	5.8	
	8		40.70	90.58	3.9725	44.8	6.0331	92.05	126.7	8.24	61.4	1.8	10.8	1.0	2.9	3.4	—	2.5	4.0	0.3	6.0	
	Malt 10		39.36	92.52	4.2656	44.1	7.2566	93.25	121.7	8.41	63.5	2.0	11.6	1.2	3.1	3.1	—	2.6	4.2	—	6.0	415.5
	13		38.85	91.76	4.1081	43.5	9.3831	91.82	147.5	8.32	64.3	2.1	12.0	1.1	3.1	3.8	—	2.7	4.3	0.4	6.0	
	Steep	Gold. barley	41.84	91.61	4.3085	41.5	—	—	—	0.71	59.7	0.8	2.7	0.8	1.2	1.3	1.4	1.1	1.0	—	5.9	
	8		40.13	88.63	3.4032	30.0	5.8063	90.06	122.9	8.06	62.3	2.1	9.8	0.7	2.8	3.1	3.3	2.2	3.7	—	5.9	
	Malt 10		37.80	91.84	4.0747	37.5	7.5909	92.32	127.1	8.30	62.3	2.2	10.4	0.8	2.7	3.0	3.7	2.0	4.0	0.3	5.9	397.9
	13		39.13	88.26	3.5943	32.5	10.8564	89.40	157.8	8.01	63.9	2.5	10.2	0.7	2.7	3.4	3.6	2.0	3.6	—	5.8	

[1]) = days of 24 hours.

TABLE VI. **Year 19**

Sort		After conclusion of normal steeping, germinated at 14–16° in — days	A	Y	Gx	R	I	S	A_I	A_{II}	D	E	F	G	H
			10 000 corns gave												
			g.	g.	g.	g. loss			g. nitrogen in the form						
			dry matter	unaffected matter (husk)	extract	as rootlets	by oxidation	Acid ≙ cc. n. NaOH	Albumin		peptic decomposition products = P			tryptic decomposition products = T	
									I	II	Denucleïn	Propepton	Pepton	Ammonia	Amine-Amide
Hannchen	Steep	0	397.3	369.9	27.4	—	—	15.5	0.22	0.08	0.08	0.01	0.02	0.01	0.2
	Malt	8	367.9	67.4	300.5	14.7	14.7	28.4	0.29	0.20	0.17	0.04	0.14	0.03	1.4
		10	360.7	60.4	300.3	16.2	20.4	29.2	0.15	0.33	0.18	0.02	0.20	0.03	1.4
		13	354.2	59.4	294.8	17.5	25.6	31.4	0.18	0.28	0.22	0.07	0.17	0.03	1.4
Chevallier II	Steep	0	409.1	385.1	24.0	—	—	16.3	0.15	0.12	0.06	0.03	0.05	0.01	0.1
	Malt	8	381.3	77.5	303.8	16.8	11.0	30.7	0.18	0.22	0.22	0.06	0.21	0.03	1.4
		10	370.9	75.9	295.0	19.0	19.2	31.5	0.13	0.23	0.24	0.07	0.20	(0.02)	1.3
		13	359.5	73.3	286.2	22.0	27.6	32.2	0.13	0.22	0.27	0.09	0.15	(0.03)	1.5
Swan's neck	Steep	0	443.4	411.2	32.2	—	—	21.2	0.22	0.08	0.08	0.03	0.08	0.20	
	Malt	8	403.6	77.4	326.2	18.3	21.5	43.0	0.25	0.33	0.27	0.05	0.28	0.05	2.0
		10	398.7	76.1	322.6	21.9	22.8	44.5	0.22	0.29	0.29	0.10	0.34	2.00	
		13	391.2	74.1	317.1	22.8	29.4	47.4	0.24	0.19	0.42	0.19	0.19	0.05	2.2
Primus	Steep	0	468.8	436.1	32.7	—	—	22.6	0.23	0.00	0.12	0.00	0.05	0.01	0.2
	Malt	8	445.8	81.0	364.8	15.1	7.9	40.6	0.34	0.36	0.20	0.00	0.25	00.4	1.5
		10	437.3	82.7	354.6	17.6	13.9	41.0	0.30	0.40	0.19	0.00	0.32	0.04	1.5
		13	419.5	71.5	348.0	18.8	30.5	40.9	0.31	0.31	0.23	0.07	0.24	1.73	
Princess	Steep	0	405.2	379.8	25.4	—	—	14.3	0.20	0.02	0.08	0.05	—	—	—
	Malt	8	369.0	73.0	296.0	17.9	18.3	29.3	0.23	0.22	0.21	0.12	—	0.03	—
		10	364.2	72.4	291.8	21.9	19.1	30.9	0.26	0.31	0.11	0 00	—	—	—
		13	356.4	71.5	284.9	22.7	26.1	33.0	0.24	0.19	0.24	0.16	—	0.04	—
Golden barley	Steep	0	383.3	359.1	24.2	—	—	13.4	0.19	0.05	0.04	0.02	0.03	0.30	
	Malt	8	355.7	70.3	285.4	17.1	10.5	33.7	0.16	0.26	0.21	0.07	0.04	1.46	
		10	347.2	70.7	276.5	20.9	15.2	35.3	0.18	0.20	0.23	0.07	0.15	0.03	1.4
		13	345.4	69.2	276.2	24.1	13.8	39.1	0.15	0.13	0.31	0.16	0.04	1.45	
Analytical error			2.1		2.6	2.4		3.3	0.07						

ley from Svalöf.

O	n	Q = n ÷ N	N	P	T	P+T		α	Ds	Cv	Pa	Ta	Aml	Rn	Ps	Ex	R′
									Numerical value of . . . process								
									(1)	(2)	(3)	(4)	(5)	(6)			
peptic bound nitrogen	Total of nitrogen	insoluble compounds	soluble decomposition products	peptic	tryptic	proteolytic	pH.	10 000 steeped corns contain — g. nitrogen in form of albumin II	Proteid solution	Albumin conversion	Peptic action	Tryptic action	Ammonia liberation	Root formation	Peptic action	% extract of dry malt stuff	g. rootlets for 100 g. malt grains
12	5.66	5.03	0.63	0.11	0.22	0.33	5.9	0.08	—	—	—	—	—	—	—	6.89	—
67	5.11	2.83	2.28	0.35	1.44	1.79	6.0		2.20	2.08	2.01	1.77	0.57	0.55	0.55	81.67	4.0
66	5.04	2.67	2.37	0.40	1.49	1.89	6.0		2.36	2.11	2.18	1.89	0.64	0.62	0.54	83.25	4.5
66	5.16	2.76	2.40	0.46	1.48	1.94	6.0		2.27	2.07	2.11	1.76	0.52	0.50	0.54	83.22	4.8
20	5.94	5.33	0.61	0.14	0.20	0.34	5.9	0.12	—	—	—	—	—	—	—	5.87	—
80	5.12	2.75	2.37	0.49	1.48	1.97	5.9		2.58	2.48	2.45	2.10	0.84	0.82	0.60	79.50	4.4
78	5.05	2.81	2.24	0.51	1.37	1.88	5.9		2.52	2.41	2.43	2.06	0.90	0.89	0.58	79.49	5.1
75	5.06	2.67	2.39	0.51	1.53	2.04	5.9		2.66	2.56	2.58	2.21	0.90	0.88	0.55	79.61	5.9
16	7.34	6.65	0.69	0.19	0.20	0.39	5.8	0.08	—	—	—	—	—	—	—	7.27	—
13	6.53	3.29	3.24	0.60	2.06	2.66	5.9		3.36	3.11	3.08	2.67	—	0.81	0.97	80.83	4.5
10	6.36	3.12	3.24	0.73	2.00	2.73	5.9		3.53	3.32	3.32	2.78	—	0.98	0.94	80.91	5.5
15	6.37	2.88	3.49	0.80	2.26	3.06	5.9		3.77	3.66	3.64	3.03	—	0.97	0.99	81.06	5.8
16	6.87	6.17	0.70	0.17	0.30	0.47	5.8	0.00	—	—	—	—	—	—	—	6.97	—
85	6.33	3.56	2.77	0.45	1.62	2.07	5.9		2.61	2.25	2.14	1.86	0.57	0.54	0.69	81.86	3.3
80	6.24	3.45	2.79	0.51	1.58	2.09	5.9		2.72	2.32	2.25	1.91	0.66	0.63	0.64	81.10	4.0
82	6.03	3.14	2.89	0.54	1.73	2.27	5.9		3.03	2.72	2.64	2.27	—	0.84	0.66	82.95	4.4
18	6.62	6.02	0.60	—	—	0.38	5.8	0.02	—	—	—	—	—	—	—	6.27	—
90	6.43	3.97	2.46	—	—	2.01	6.0		2.05	1.85	1.82	—	—	0.19	0.72	80.23	4.8
93	5.70	3.14	2.56	—	—	1.99	6.0		2.88	2.59	2.53	—	—	0.92	0.75	80.13	6.0
93	5.64	3.03	2.61	—	—	2.18	6.0		2.99	2.82	2.78	—	—	0.98	0.75	79.93	6.0
23	5.64	5.01	0.63	0.09	0.30	0.39	5.9	0.05	—	—	—	—	—	—	—	6.31	—
83	4.95	2.75	2.20	0.32	1.46	1.78	5.9		2.26	2.05	2.08	1.85	—	0.69	0.60	80.24	4.7
90	4.87	2.53	2.34	0.45	1.51	1.96	5.9		2.48	2.33	2.34	1.98	—	0.77	0.67	79.67	6.0
79	4.96	2.72	2.24	0.51	1.45	1.96	5.8		2.29	2.21	2.25	1.83	—	0.68	0.56	79.98	6.9
	0.13	0.12	0.05	0.07			0.1		0.07								

TABLE VII. **Year 1913. Barley from Svalöf. For 1000 g. dry matter:**

Columns marked			R_1	Gx_1	S_1	n_1	N_1	A_{I_1}	A_{II_1}	P_1	T_1	P_1+T_1	O_1
Sort		After conclusion of normal steeping germinat. at 14-16° in — days	g. rootlets	g. extract	Acid ∾ cc. n. NaOH	g. nitrogen in form of: total nitrogen	soluble compounds	Albumin: I	Albumin: II	decomposition products: peptic	decomposition products: tryptic	decomposition products: proteolytic	peptide bound nitrogen
Hannchen	Steep	0	—	67.0	37.9	13.83	1.54	0.54	0.20	0.27	0.54	0.81	0.29
	Malt	8	35.9	734.4	69.4	12.49	5.57	0.71	0.49	0.86	3.52	4.38	1.64
		10	39.6	733.9	71.4	12.32	5.79	0.37	0.81	0.98	3.64	4.62	1.61
		13	42.8	720.5	76.7	12.61	5.87	0.44	0.68	1.12	3.62	4.74	1.61
Chevallier II	Steep	0	—	57.0	38.7	14.11	1.45	0.36	0.29	0.33	0.48	0.81	0.48
	Malt	8	39.9	721.5	72.9	12.16	5.63	0.43	0.52	1.16	3.52	4.68	1.90
		10	45.1	700.6	74.8	11.99	5.32	0.31	0.55	1.21	3.25	4.46	1.85
		13	52.3	679.7	76.5	12.02	5.68	0.31	0.52	1.21	3.63	4.84	1.78
Swan's neck	Steep	0	—	72.1	47.4	16.43	1.54	0.49	0.18	0.43	0.45	0.88	0.36
	Malt	8	41.0	730.0	96.2	14.61	7.25	0.56	0.74	1.34	4.61	5.95	2.53
		10	49.0	722.0	99.6	14.23	7.25	0.49	0.65	1.63	4.48	6.11	2.46
		13	51.0	709.7	106.1	14.26	7.81	0.54	0.43	1.79	5.06	6.85	2.57
Primus	Steep	0	—	68.6	47.4	14.42	1.47	0.48	0.00	0.36	0.63	0.99	0.34
	Malt	8	31.7	765.7	85.2	13.29	5.81	0.71	0.76	0.94	3.40	4.34	1.78
		10	36.9	744.3	86.1	13.10	5.86	0.63	0.84	1.07	3.32	4.39	1.68
		13	39.5	730.5	85.8	12.66	6.07	0.65	0.65	1.13	3.63	4.76	1.72
Princess	Steep	0	—	61.1	34.4	15.93	1.44	0.48	0.05	—	—	0.91	0.43
	Malt	8	43.1	712.5	70.5	15.48	5.92	0.55	0.53	—	—	4.84	2.17
		10	52.7	702.4	74.4	13.72	6.16	0.63	0.75	—	—	4.79	2.24
		13	54.6	685.8	79.4	13.58	6.28	0.58	0.46	—	—	5.26	2.24
Golden barley	Steep	0	—	60.8	33.7	14.17	1.58	0.48	0.13	0.23	0.75	0.98	0.58
	Malt	8	43.0	717.2	84.7	12.44	5.53	0.40	0.65	0.80	3.67	4.47	2.09
		10	52 5	694.8	88.7	12.24	5.88	0.45	0.50	1.13	3.79	4.92	2.26
		13	60.6	694.1	98.3	12.46	5.63	0.38	0.33	1.28	3.64	4.92	1.99
Analytical error c.			6.4	6.9	8.7	0.34	0.13	0.19					

Found for values		a	b	m	c	p	s	q	k	U	h	e	f						g				
Sort		After conclusion of normal steeping germinated at 14—16° in . . . days[1]	1000 wet corns weigh gr.	% dry matter in the corns	gr. hydrous matter	containing nitrogen ≈ cc. n/10 Acid	Quantity of rootlet: Root: gr. rootlets	Quantity of rootlet: Root: with % dry matter	Quantity of rootlet: Corn: gr. malt grains	% extract (Ball.)	25 cc dilute wort ≈ no. of corns	25 cc. dilute wort contains: Acid ≈ cc. n/10 NaOH	25 cc. dilute wort contains: Nitrogen ≈ cc. n/10 Acid as: total nitrogen	precipitable by: $SnCl_2$	$HgCl_2$	$FeAc_3$	$UrAc_2$	$MgSO_4$	formol-titratable	50 cc. dilute wort contains nitrogen in form of ammonia ≈ cc. n/10 acid	p_H	10 000 barley corns contain g. dry matter	% dry matter in the barley corns	Normal steeping time of the sample in hours
Hannchen	Steep	0	41.94	92.35	4.0127	53.1	—	—	—	0.83	59.6	1.3	3.3	1.5	1.8	2.1	2.1	1.6	0.9	0.2	5.7			
	Malt	8	37.72	92.19	4.0860	52.5	5.6897	90.90	135.0	8.43	66.3	2.8	13.4	2.6	4.5	5.0	5.7	3.8	4.1	0.4	5.9	393.4	89.11	48
		10	37.78	92.24	4.0200	52.0	7.6230	90.31	146.8	8.36	66.2	2.7	13.5	2.2	4.6	4.8	5.7	3.8	3.9	0.4	5.9			
		13	36.03	92.06	4.1662	54.7	9.2441	91.27	146.5	8.38	69.4	2.7	13.7	2.2	4.6	5.1	6.0	3.8	4.0	—	5.9			
Chevall. II	Steep	0	44.68	92.41	4.0147	56.8	—	—	—	0.82	56.0	1.4	3.6	1.6	1.8	2.2	2.2	1.7	0.9	0.1	5.9			
	Malt	8	40.46	91.05	4.0092	56.4	5.3703	90.14	127.9	8.17	61.8	2.9	15.4	2.6	4.3	5.2	5.9	3 7	5.2	0.5	5.9	417.1	88.61	72
		10	40.02	91.45	4.0310	56.5	7.4192	90.97	138.5	8.09	62.5	3.0	14.6	2.7	4.3	5.2	6.0	3.6	4.6	0.5	5.9			
		13	39.07	89.99	4.0023	56.5	9.5143	91.13	141.2	7.98	64.0	3.1	14.5	2.7	4.5	5.5	5.9	3.8	4.4	0.5	5.9			
Swan's neck	Steep	0	42.93	93.28	4.0493	59.8	—	—	—	0.78	58.2	1.2	3.8	1.7	2.2	2.3	2.4	1.9	1.2	0.1	5.9			
	Malt	8	37.47	92.42	4.0644	58.3	7.4139	90.99	113.9	8.16	66.7	3.2	18.0	1 4	4.6	5.4	6.5	4.0	6.3	0.7	5.8			
		10	36.28	91.54	4.0500	58.1	10.6858	93.25	120.6	8.09	68.9	3.6	19.0	1.4	4.6	5.4	6.8	3.7	6.7	0.8	5.8	404.5)	88.5)[2]	72
		13	36.12	88.45	4.0367	57.4	11.5943	92.01	120.9	7.88	69.2	4.0	20.2	1.4	4.4	5.2	6.8	3.4	7.3	0.7	5.7			
Primus	Steep	0	44.42	93.20	4.0203	55.8	—	—	—	0.84	56.3	1.3	3.5	1.4	1.7	2.1	2.1	1.4	1.0	0.2	5.7			
	Malt	8	39.76	92.33	4.0595	55.3	4.7381	89.43	115.4	8.19	62.9	2.8	13.9	1.5	4.3	4.7	6.2	3.7	4.4	0.6	5.8	425.5	88.46	96
		10	38.19	91.62	4.0297	53.7	7.7949	91.65	110.9	8.27	65.5	3.2	16.3	1.6	4.4	4.8	6.3	3.6	5.4	0.8	5.8			
		13	39.55	89.45	4.0790	56.3	8.4159	90.43	140.8	8.04	63.2	3.5	16.5	1.4	4.4	4.8	6.2	3.5	5.6	0.9	5.7			
Princess	Steep	0	42.61	93.22	4.0265	53.7	—	—	—	0.85	58.7	1.3	3.8	1.4	2.3	2.3	2.3	1.8	0.9	0.1	5.7			
	Malt	8	40.38	89.96	4.0225	52.8	5.0258	90.54	120.5	7.95	61.9	2.8	12.9	1.6	4.5	4.2	5.3	3.4	3.9	0.5	5.9	408 4	88.00	96
		10	37.09	90.81	4.0167	53.0	8.3726	90.92	109.7	8.10	67.4	3.2	15.0	1.5	4.6	4.3	5.7	3.4	4.8	0.6	5.9			
		13	35.41	91.77	4.0240	55.2	12.2764	90.66	122.0	8.19	70.6	3.8	16.7	1.3	4.3	4.6	5.7	3.0	5.6	0.6	5.6			
Gold. barley	Steep	0	39.28	92.62	4.0597	56.4	—	—	—	0.75	63.6	1.3	3.4	1.3	2.0	2.2	2.0	1.6	0.9	0.1	5.9			
	Malt	8	35.80	91.30	4.0149	53.9	7.8798	90.80	126.5	8.07	69.8	2.7	12.1	1.4	4.1	4.1	4.9	3.1	3.5	0.5	5.9	371.0	88.83	96
		10	34.67	91.54	4.0201	52.2	10.2653	92.25	126.8	8.10	72.1	2.8	12.2	1.4	4.0	4.0	4.8	3.1	3.5	0.5	5.8			
		13	33.55	90.39	4.0088	52.4	14.7008	91.74	145.4	7.91	74.5	2.9	12.7	1.4	4.1	4.1	5.0	3.0	3.7	0.4	5.8			

[1] = days of 24 hours. [2] Percentage of dry matter here not determined, but estimated at the value above given.

TABLE IX. **Year 19**

Sort		After conclusion of normal steeping, germinated at 14—16° in ... days	A	Y	Gx	R	I	S	A_I	A_{II}	D	E	F	G	H	O
			10 000 corns gave													
			g.	g.	g.	g. loss			g. nitrogen in the form							
			dry matter	unaffected matter (husk)	extract	as rootlets	by oxidation	Acid cc. n. NaOH	Albumin		peptic decomposition products = P			tryptic decomposition products = T		peptide-bound nitrogen
									I	II	Denucléin	Propepton	Pepton	Ammonia	Amine-Amide	
Hannchen 2 rowed	Steep	0	387.3	358.9	28.4	—	—	22.5	0.34	÷0.03	0.11	0.07	÷0.01	0.02	0.27	0.2
	Malt	8	347.7	67.2	280.5	14.5	25.1	42.3	0.55	0.16	0.24	0.10	0.15	0.04	1.60	0.8
		10	348.5	70.0	278.5	17.7	21.1	40.4	0.46	0.29	0.22	0.05	0.19	0.04	1.60	0.8
		13	331.7	65.5	266.2	20.8	34.8	38.9	0.44	0.22	0.27	0.11	0.16	1.56		0.8
Chevallier II 2 rowed	Steep	0	412.9	383.1	29.8	—	—	24.5	0.41	÷0.02	0.12	0.03	0.01	0.01	0.34	0.2
	Malt	8	368.4	77.3	291.1	15.3	29.2	47.6	0.58	0.06	0.34	0.20	0.15	0.06	2.10	1.1
		10	366.0	81.3	284.7	19.5	27.4	47.7	0.61	0.00	0.35	0.20	0.18	0.06	1.88	1.0
		13	351.6	77.2	274.4	24.0	37.3	48.3	0.59	0.01	0.38	0.23	0.09	0.06	1.81	0.9
Swan's neck 2-rowed	Steep	0	400.5	373.3	27.2	—	—	20.8	0.41	0.02	0.09	0.04	0.01	0.01	0.33	0.2
	Malt	8	346 3	77.4	268.9	22.2	32.0	48.6	0.29	0.40	0.27	0.16	0.25	0.07	2.33	1.3
		10	333.1	74.0	258.1	30.0	38.4	51.5	0.29	0.31	0.33	0.16	0.29	2.48		1.3
		13	319.5	68.8	250.7	31.9	49.1	58.1	0.28	0.25	0.37	0.16	0.31	0.07	2.64	1.4
Primus 2-rowed	Steep	0	414.0	383.6	30.4	—	—	23.8	0.34	÷0.08	0.15	0.10	0.00	0.02	0.33	0.2
	Malt	8	367.1	80.6	286.5	14.6	32.3	44.5	0.34	0.40	0.23	0.08	0.32	0.06	1.66	0.9
		10	349.9	71.6	278.3	24.6	39.5	48.9	0.35	0.35	0.24	0.08	0.33	0.09	2.05	1.1
		13	353.8	73.5	280.3	21.4	38.8	54.7	0.30	0.40	0.27	0.08	0.32	0.10	2.18	1.2
Princess 2-rowed	Steep	0	397.2	367.7	29.5	—	—	22.8	0.34	0.07	0.13	0.01	÷0.01	0.01	0.36	0.2
	Malt	8	363.2	80.7	282.5	15.2	18.8	44.6	0.36	0.45	0.20	÷0.05	0.24	0.06	1.65	0.8
		10	336.8	72.3	264.5	25.7	34.7	47.2	0.31	0.44	0.20	÷0.05	0.28	0.07	1.86	1.0
		13	324.9	69.6	255.3	32.3	40.0	53.5	0.27	0.27	0.31	0.06	0.22	0.06	2.13	1.1
Golden barley 2-rowed	Steep	0	363.8	339.8	24.0	—	—	19.8	0.29	0.04	0.11	0.03	÷0.04	0.01	0.31	0.1
	Malt	8	326.8	72.7	254.1	20.2	16 8	39.2	0.28	0.36	0.19	÷0.01	0.15	0.05	1.40	0.7
		10	317.3	70.3	247.0	25.9	20.6	38.8	0.27	0.32	0.18	0.01	0.14	0.05	1.40	0.6
		13	303.2	73.0	230.2	31.1	29.5	39.5	0.26	0.31	0.20	÷0.01	0.18	0.04	1.41	0.7
Analytical error			2.1		2.6	2.4		3.3	0.07							

rley from Svalöf.

n	Q = n÷N	N	P	T	P+T		α	Ds	Cv	Pa	Ta	Aml	Rn	Ps	Ex	R′	
								Numerical value of . . . process									
								(1)	(2)	(3)	(4)	(5)	6				
Total of nitrogen	insoluble compounds	soluble decomposition products	peptic	tryptic	proteolytic	p_H	10 000 steeped corns contain — g. nitrogen in form of Albumin II	Proteid solution	Albumin conversion	Peptic action	Tryptic action	Ammonia liberation	Root formation	Peptic action	% extract of dry malt stuff	g. rootlets for 100 g. malt grains	hussars
7.77	7.00	0.77	0.17	0.29	0.46	5.7	÷0.03	—	—	—	—	—	—	—	7.32	—	
5.79	3.95	2.84	0.49	1.64	2 13	5.9		3.05	2.86	2.65	2.33	1.00	0.98	0.67	80.67	4.2	none
5.84	3.99	2.85	0.46	1.64	2.10	5.9		3.01	2.69	2.57	2.28	0.95	0.93	0.62	79.89	5.2	none
5.63	3.87	2.76	0.54	1.56	2.10	5.9		3.13	2.88	2.78	2.41	—	1.14	0.60	80.26	6.3	some few
8.85	7.95	0.90	0.16	0.35	0.51	5.9	÷0.02	—	—	—	—	—	—	—	7.23	—	
7.98	4.49	3.49	0.69	2.16	2.85	5.9		3.46	3.38	3.21	2.68	0.92	0.87	0.96	79.03	4.2	none
7.85	4.57	3.28	0.73	1.94	2.67	5.9		3.38	3.36	3.16	2.59	1.05	1.00	0.82	77.80	5.4	some few
7.72	4.55	3.17	0.70	1.87	2.57	5.9		3.40	3.37	3.19	2.65	1.18	1.13	0.76	78.06	6.7	some
8.88	7.97	0.91	0.14	0.34	0.48	5.9	0.02	—	—	—	—	—	—	—	6.80	—	
7.53	3.76	3.77	0.68	2.40	3 08	5.8		4.21	3.83	3.95	3.41	1.41	1.35	1.03	77.66	6.5	few
7.29	3.43	3.86	0.78	2.48	3.26	5.8		4.54	4.25	4.37	3.73	—	1.59	1.08	77.73	8.9	many
7.19	3.11	4.08	0.84	2.71	3.55	5.7		4.86	4.63	4.76	4.06	1.75	1.69	1.19	78.47	9.6	many
8.63	7.77	0.86	0.25	0.35	0.60	5.7	÷0.08	—	—	- -	—	—	—	—	7.34	—	
7.58	4.49	3.09	0.63	1.72	2.35	5.8		3.28	2.80	2.80	2.42	1.09	1.05	0.74	78.05	4.1	none
7.12	3.63	3.49	0.65	2.14	2.79	5.8		4.14	3.71	3.70	3.30	1.58	1.51	0.91	79.55	7.0	a good few
7.64	3.99	3.65	0.67	2.28	2.95	5.7		3.78	3.30	3.34	2.92	1.07	0.99	1.01	79.22	6.0	fairly numerous
7.96	7.05	0.91	0.13	0.37	0.50	5.7	0.07	—	—	—	—	—	—	—	7.42	—	
7.42	4.51	2.91	0.39	1.71	2.10	5.9		2.54	2.16	2.14	1.88	0.59	0.54	0.66	77.78	4.2	some
6.85	3.74	3 11	0.43	1.93	2.36	5.9		3.31	2.94	2.97	2.67	1.17	1.11	0.79	78.54	7.6	many
6.80	3.48	3.32	0.59	2.19	2.78	5.6		3.57	3.37	3.44	2.98	1.21	1.16	0.88	78.57	10.1	many
7.64	6.89	0.75	0.10	0.32	0.42	5.9	0.04	—	—	—	—	—	—	—	6.59	—	
6.73	4.31	2.42	0.33	1.45	1.78	5.9		2.58	2.26	2.27	2.04	0.94	0.91	0.52	77.75	6.2	some few
6.30	3.93	2.37	0.33	1.45	1.78	5.8		2.96	2.68	2.70	2.47	1.38	1.34	0.49	77.86	8.1	a good few
6 14	3.75	2.39	0.37	1.45	1.82	5.8		3.14	2.87	2.90	2.63	1.53	1.50	0.51	76.93	10.1	many
0.13	0.12	0.05		0.07		0.1					0.07						

TABLE X. **Year 1914. Barley from Svalöf. For 1000 g. dry matter:**

Sort		After conclusion of normal steeping, germinat. at 14-16° in — days	R_1 g. rootlets	Gx_1 g. extract	S_1 Acid ∞ cc. n. NaOH	n_1 g. nitrogen in form of: total of nitrogen	N_1 soluble compounds	A_{I_1} Albumin I	A_{II_1} Albumin II	P_1 decomposition products: peptic	T_1 tryptic	P_1+T_1 proteolytic	O_1 peptide-bound nitrogen
Hannchen	Steep	0	—	72.2	57.2	19.75	1.96	0.86	÷ 0.08	0.43	0.74	1.17	0.51
	Malt	8	36.9	713.0	107.5	17.26	7.22	1.40	0.41	1.25	4 17	5.42	2.21
		10	45.0	707.9	102 7	17.39	7.24	1.17	0.74	1.17	4.17	5.34	2.08
		13	52.9	676.7	98.9	16.85	7.02	1.12	0.56	1.37	3.97	5.34	2.03
Chevallier II	Steep	0	—	71.5	58 8	21.22	2.16	0.98	÷ 0.05	0.38	0.84	1.22	0.50
	Malt	8	36.7	698.1	114.1	19.14	8.37	1.39	0.12	1.65	5.18	6.83	2.81
		10	46.8	682.7	114.4	18.82	7.87	1.46	0.00	1.75	4.65	6.40	2.47
		13	57.6	658.0	115.8	18.51	7.60	1.41	0.02	1.68	4.48	6 16	2.33
Swan's neck	Steep	0	—	67.2	51.4	21.95	2.25	1.01	0.05	0.35	0.84	1.19	0.69
	Malt	8	54.9	664.7	120.1	18.61	9.32	0.72	0.99	1.68	5.93	7.61	3.24 [1]
		10	74.2	638.0	127.3	18.02	9.54	0.72	0.77	1.93	6.13	8.06	3.36
		13	78.9	619.7	143.6	17.77	10.09	0.69	0.62	2.08	6.70	8.78	3.63
Primus	Steep	0	—	71.4	55.9	20.28	2.02	0.80	÷ 0.19	0 59	0.82	1.41	0.56
	Malt	8	34.3	673.3	104.6	17.81	7.26	0.80	0.94	1.48	4.04	5.52	2.30
		10	57.8	654.0	114.9	16.73	8.20	0.82	0.82	1.53	5.03	6.56	2.70
		13	50.3	658.7	128.5	17.95	8.58	0.71	0.94	1.57	5.36	6.93	2.94
Princess	Steep	0	—	72.2	55.8	19.49	2.23	0.83	0.17	0.32	0.91	1.23	0.54
	Malt	8	37.2	691.8	109.2	18.17	7.13	0.88	1.10	0.96	4.19	5.15	2.16
		10	62.9	647.8	115.6	16.78	7.62	0.76	1.08	1.05	4.73	5.78	2.47
		13	79.1	625.2	131.0	16.65	8.13	0.66	0.66	1.44	5.36	6.80	2.69
Golden barley	Steep	0	—	64.7	53.4	20.59	2.02	0.78	0.11	0.27	0.86	1.13	0.51
	Malt	8	54.4	684.8	105.6	18.14	6.52	0.75	0.97	0.89	3.91	4.80	1.91
		10	69.8	665.7	104.6	16.98	6.39	0.73	0.86	0.89	3.91	4.80	1.83
		13	83.8	620.4	106.5	16.55	6.44	0.70	0.84	1.00	3.91	4.91	1.89
Analytical error			6.4	6.9	8.7	0.34	0.13	0.19					

[1])

between the values found and the quantity of acid titrated. The wort is thus, as was to be expected, rich in buffers. The titrated acids are probably for the most part weak; titration was made to a concentration of hydrogen ions about $10^{\div 3.1}$.

The total quantity of nitrogen in the grain differs greatly from year to year, which must chiefly be due to the varying amount of nitrogenous matter contained in the soil; in all six races, the values are lowest for the crops from 1913 and highest for those of 1914. The quantities of soluble nitrogen compounds in the wort produced likewise differ from year to year, but not nearly to so marked a degree as the total quantity of nitrogen in the grain. The years 1912 and 1913 show no pronounced difference in the amount of soluble nitrogen compounds, whereas the 1914 crop is distinctly richer in these.

Only in a single instance, that of Chevalier II from 1914, was the wort produced free from Albumin II; in all other cases, this group was more or less richly represented. The requisite equilibrium between the proteïn solution process (1) and the albumin conversion process (2) was thus in most cases not attained. The ratio between the protein solution process (1) and peptase action (3) was by no means in all cases as desired; i. e. $(1) \leqq (3)$; not even in the case of Chevallier II from 1914, where the albumin conversion process was complete; in this instance, the quantity of Albumin I was very considerable.

Swan's neck is on the whole richer in nitrogen than are the others; it is also especially rich in soluble nitrogen compounds and proteolytic decomposition products, which must probably be regarded as due to a particularly high content of proteolytic enzymes. No such abundance of the latter is apparent in Golden barley, the soluble nitrogen compounds being here, together with the proteolytic decomposition products, more or less uniform for the three years, despite the fact that the total quantity of nitrogen varies greatly from year to year.

Section II. Samples from Tystofte and Abed Experimental Stations.

From the experimental stations Tystofte and Abed, we have samples from the years 1913 and 1914 comprising the four two-rowed races

Tystofte prentice, Svalöf Golden barley, Binder barley (Abed 411), and Abed 570,

and the two six-rowed:

Tystofte Cross barley and Abed 110 (Abed July barley).

In the material from Abed, however, Tystofte Cross barley was lacking, and Abed 110 from here was not grown at the experimental station itself, but near Nakskov, in the vicinity

From Tystofte, we have for 1913, two sets of samples of all the two-rowed races; the first lot, marked A, had suffered some damage during harvesting; the latter, marked F, had been harvested under favourable conditions. For 1914, there were three samples of Tystofte Prentice, of which one, No. 3. was grown on the same ground as the remaining sorts, whereas Nos. 1 and 2 came from another soil, evidently less rich in nitrogen. Of these two last named samples, No. 1 had been manured with 400 kg. magnium sulphate per hectare; otherwise, they had been treated alike.

As was to be expected from the state of the weather at harvest time, the normal steeping period is far longer for the samples marked F than for those marked A. On the whole, the values for vitality and steeping time are somewhat lower for the 1914 samples than for those reaped under favourable conditions in 1913.

In the Abed samples from 1913, vitality and steeping time are very unfavourable, owing to bad harvest weather.

On the whole, the weight of the grain was a good deal lower for the 1914 samples than for those of 1913, owing to the far shorter ripening period in the former year.

As is invariably the case, the two-rowed sorts are larger-grained than the six-rowed, and of these last, the Tystofte Cross barley appears distinctly larger than Abed 110. The weight of grain in the two-rowed sorts, on the other hand, appears to be uniform.

Root development was in all cases abundant for the two-rowed sorts; as a rule, there was well over 2 g. for 100 g. malt grain. Possibly the process takes place somewhat more rapidly in Tystofte Prentice and Binder barley than in Golden barley and Abed 570. Of the six-rowed sorts, only the Tystofte Cross barley from Tystofte 1914 failed to reach the

TABLE XI. **Barley from Tystofte.**

Harvest	1913 A							
Locality	Tystofte experimental station							
Soil								
Weather	unfavourable							
Sort	Tystofte Prentice		Svalöf Golden barley		Binder barley (Abed 411)		Abed 570	
% of germinative	energy	capacity	energy	capacity	energy	capacity	energy	capacity
Steeped for 6	80.2	90.8	92.6	97.2	76.6	94.2	72.0	88.2
. . . hours 24	76.4	89.6	94.0	98.4	91.2	96.2	79.4	85.4
in distilled 48	74.8	88.2	91.4	94.6	90.8	96.8	75.6	81.2
water at 72	64.4	81.0	89.4	94.8	86.2	92.8	69.0	82.0
18°—20° 96	36.2	60.0	86.6	93.2	80.0	91.0	56.0	71.2
Analytical error	2 %							

Harvest	1913 F											
Locality	Tystofte experimental station											
Soil												
Weather	favourable											
Sort	Tystofte Prentice		Svalöf Golden barley		Binder barley (Abed 411)		Abed 570		Tystofte Cross barley		Abed 110	
% of germinative	energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity
Steeped for 6	98.0	98.8	96.4	99.2	98.8	99.4	97.8	98.8	92.2	99.0	96.0	99.2
. . . hours 24	97.2	99.2	95.6	97.6	98.6	99.2	97.4	98.0	96.2	99.2	98.4	99.8
in distilled 48	96.4	99.0	96.2	98.2	97.2	98.2	97.6	98.0	93.4	98.2	98.4	99.6
water at 72	92.2	97.8	95.2	98.0	96.2	98.2	93.6	96.8	91.6	96.2	98.4	99.2
18°—20° 96	81.0	94.6	88.8	98.2	83.2	94.4	79.4	95.2	88.6	96.2	96.0	98.0
Analytical error	2 %											

Harvest	1914															
Locality	Tystofte experimental station															
Soil																
Weather	Brief ripening period owing to summer drought															
Sort	Tystofte Prentice with $Mg.SO_4$				Tystofte Prentice without $Mg.SO_4$				Svalöf Golden barley		Binder barley (Abed 411)		Abed 570		Tystofte Cross barley		Abed 110	
% of germinative	energy		capacity		energy		capacity		energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity
Steeped for 6	99.6	99.6	99.4	99.6	98.0	99.2	88.8	94.8	96.8	87.8	98.0	98.6	95.4	95.8	81.8	85.0
. . . hours 24	99.6	99.6	99.6	99.6	97.4	98.8	86.6	88.6	93.2	97.6	96.2	97.6	92.8	95.0	79.0	79.2
in distilled 48	99.2	99.6	99.6	99.6	97.2	97.6	84.6	86.8	93.0	94.4	93.0	97.0	89.4	90.0	78.0	82.8
water at 72	99.4	99.6	99.0	99.6	95.4	98.2	90.0	95.0	91.0	96.6	82.8	94.4	86.8	91.8	72.6	77.4
18°—20° 96	99.6	99.6	98.6	99.2	95.4	97.4	83.8	86.4	65.4	90.4	75.2	84.6	83.2	87.0	67.4	69.0
Analytical error	2 %															

TABLE XII. **Barley from Abed.**

Harvest		1913										
Locality		Abed experimental station								near Nakskov		
Soil		Medium strong clay mould, somewhat coldish soil								strong clay mould		
Weather		unfavourable										
Sort		Tystofte Prentice		Svalöf Gold. barley		Bind. barley (Abed 411)		Abed 570		Abed 110		
% of germinative		energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity	
Steeped for	6	78.2	84.2	78.2	85.2	78.2	85.0	82.2	89.4	82.0	89.4	
. . . hours	24	80.8	86.0	81.2	87.0	79.6	87.0	84.0	89.8	80.6	88.6	
in distilled	48	79.6	83.6	78.8	85.0	79.0	84.8	78.8	84.0	81.0	88.2	
water at	72	77.6	83.8	73.0	82.0	74.0	81.8	77.6	88.0	75.6	84.8	
18—20°	96	61.2	75.0	60.8	76.8	52.8	77.2	27.4	56.8	74.6	85.8	
Analytical error		2 %										

Harvest		1914										
Locality		Abed experimental station								near Nakskov		
Soil		Medium strong clay mould, somewhat coldish soil								strong clay mould		
Weather		Brief ripening period										
Sort		Tystofte Prentice		Svalöf Gold. barley		Bind. barley (Abed 411)		Abed 570		Abed 110		
% of germinative		energy	capacity	energy	capacity	energy	capacity	energy	capacity	energy	capacity	
Steeped for	6	98.8	99.8	98.4	98.6	99.6	99.6	98.2	98.6	89.4	89.4	
. . . hours	24	98.4	99.2	96.8	99.0	99.6	99.8	97.2	98.4	87.8	88.6	
in distilled	48	96.2	98.4	97.4	98.8	99.4	99.4	97.4	97.4	87.2	87.4	
water at	72	97.6	98.2	95.2	96.6	97.6	99.2	90.4	97.4	87.6	88.2	
18—20°	96	94.0	97.6	90.0	96.8	96.2	97.4	91.6	97.4	81.0	82.0	
Analytical error		2 %										

mentioned value here; only 2,1 % rootlets, at the outside, being formed.

The quantity of extract exceeded in all cases 73 % of the dry malt stuff, even after the shortest germinating periods. No racial difference was discernible in this respect.

The values for concentration of hydrogen ions lay within the limits $10 \div 5.6$ and $10 \div 6.2$, very close to those found for the Svalöf samples. Here also they were independent of the quantity of acid titrated.

The total quantity of nitrogen varied considerably for one and the same sort from year to year, a difference being also noticeable between the two localities; even with the Prentice

TABLE XIII. **Year 1913. Barley from Tystofte. A.**

Found for value			a	b	m	c	p	s	q	k	U	h	e	f						g				
Sort		After conclusion of normal steeping germinated at 14—16° in . . . days[1])	1000 wet corns weigh gr.	% dry matter in the corns	gr. hydrous matter	∾ containing nitrogen cc. n/10 acid	Quantity of rootlet: Root gr. rootlets	Quantity of rootlet: Root with % dry matter	Quantity of rootlet: Corn gr. malt grains	% extract (Ball.)	25 cc. dilute wort ∾ no of corns	25 cc. dilute wort contains: acid ∾ cc. n/10 NaOH	25 cc. dilute wort contains: nitrogen ∾ cc. n/10 acid as: total nitrogen	precipitable by: $SnCl_2$	$HgCl_2$	$FeAc_3$	$UrAc_2$	$MgSO_4$	formol-titratable	50 cc. dilute wort contains nitrogen in form of ammonia ∾ cc. n/10 acid	p_H	10 000 barley corns contain g. dry matter	% dry matter in the barley corns	Normal steeping time of the sample in hours
Tystofte Prentice 2-rowed	Steep	0	48.40	91.40	4.0525	46.5	—	—	—	0.59	51.7	1.4	2.3	1.0	1.6	1.7	1.9	1.4	0.9	0.1	5.8	446.6	89.59	48
	Malt	8	45.52	90.61	4.0326	45.3	3.9643	92.68	123.6	8.11	54.9	2.3	11.6	1.6	3.6	4.0	4.8	2.8	3.7	—	6.0			
		10	43.35	92.19	4.3036	49.0	5.7283	91.42	118.2	8.33	57.7	2.5	12.7	1.1	3.6	3.9	4.9	2.8	4.2	0 4	6.0			
		13	42.49	91.93	4.1175	47.5	6.9962	92.73	124.9	8.24	58.8	2.6	12.8	1.2	3.6	4.3	4.8	2.8	4.3	—	6.0			
Svalöf Golden barley 2-rowed	Steep	0	44.88	90.49	4.0072	47.5	—	—	—	0.82	55.7	1.0	3.0	1.3	1.6	1.7	1.8	1.3	0.7	—	6.0	418.2	89.56	48
	Malt	8	41.78	90.01	4.1287	47.5	4.2272	91.46	134.3	8.03	59.8	2.0	9.7	1.5	3.3	3.6	4 0	2.7	3.2	—	6.0			
		10	40.29	92.36	4.4026	51.0	5.0670	92.86	129 7	8.23	62.0	2.0	10.9	1.3	3.3	3.6	4.2	2.6	3.4	—	6.0			
		13	39.95	92.00	4.0305	47.0	5.7958	93.24	124.6	8.20	62.6	2.0	11.2	1.1	3.6	3.4	4.3	2.7	3.5	—	6.0			
Binder barley (Abed 411) 2-rowed	Steep	0	45.77	92.19	4.1160	50.5	—	—	—	0.77	54.6	1.1	3.5	1.1	1.9	1.9	2.0	1.5	(1.9)	0.2	6.0	437.3	88.34	48
	Malt	6	43.58	89.17	4.2735	51.5	4.6620	89.88	127.7	7.98	57.4	2.3	12 7	1.2	3.6	3.8	4.6	2.8	4.1	—	6.0			
		8	40.68	91.46	4.3463	52.5	6.8831	91.82	118.6	8.21	61.5	2.4	13.1	1.1	3.7	3.9	4.7	2.8	4.3	—	6.0			
		11	40.22	88.50	4.1487	50.0	9.2498	90.01	132.4	7.90	62.2	2.7	13.8	1.0	3.8	4.2	5.1	2.9	4.2	0.5	5.9			
Abed 570 (Imp. Type) 2-rowed	Steep	0	45.51	91.96	4.3225	53.5	—	—	—	0.83	54.9	1.3	3.2	0.9	1.7	1.7	1.7	1.3	0.7	0.2	6.1	430.1	88.16	24
	Malt	6	42.47	90.88	4.2300	51.0	3.6897	90.94	143.4	8.14	58.9	2.2	11.3	1.2	3.7	3.7	4.4	2.8	3.3	—	6.2			
		8	40.81	92.11	4.0040	48.5	4.7476	92.82	136.5	8.26	61.3	2.2	11.5	1.4	3.9	3.9	4.6	2.9	3.3	—	6.2			
		11	41.81	91.71	4.2620	53.0	4.6670	92.10	137.0	8.24	59.8	2.2	11.3	1.3	3.8	3.8	4.8	3.0	3.1	0.4	6.2			

[1]) = days of 24 hours.

TABLE XIV. **Barley fron**

Columns marked		A	Y	Gx	R	I	S	A_I	A_{II}	D	E	F	G	H	O
Sort	After conclusion of normal steeping germinated at 14—16° in — days	10 000 corns gave													
		g. dry matter	g unaffected matter (husk)	g Extract	g. loss as rootlets	g. loss by oxidation	Acid ∞ n. NaOH	g. nitrogen in the form of Albumin I	Albumin II	peptic decomposition products = P Denucleïn	Pro-pepton	Pepton	tryptic decomposition products = T Ammonia	Amine-Amide	peptide bound nitrogen
Tystofte Prentice 2-rowed	Steep 0	442.3	419.1	23.2	—	—	27.1	0.27	0.08	0.08	0.03	0.06	0.01	0.09	0.24
	8	412.4	87.3	325.1	13.5	20.4	41.9	0.41	0.20	0.31	0.10	0.20	1.74		0.94
	Malt 10	399.7	81.5	318.2	19.2	23.4	43.4	0.27	0.33	0.27	0.08	0.24	0.04	1.85	1.02
	13	390.7	82.3	308.4	22.1	29.5	44.2	0.29	0.22	0.35	0.16	0.12	1.91		1.02
Svalöf Golden barley 2-rowed	Steep 0	406.2	376.2	30.0	—	—	18.5	0.33	÷0.03	0.10	0.03	0.02	0.30		0.18
	8	376.1	80.6	295.5	12.0	18.1	33.4	0.35	0.21	0.21	0.07	0.10	1.33		0.75
	Malt 10	372.1	80.3	291.8	14.6	19.5	32.2	0.29	0.23	0.22	0.07	0.14	1.51		0.77
	13	367.6	79.2	288.4	17.3	21.3	32.4	0.25	0.41	0.15	÷0.05	0.20	1.55		0.79
Binder barley (Abed 411) 2-rowed	Steep 0	432.0	393.3	28.7	—	—	20.2	0.28	0.10	0.11	0.00	0.02	0.03	0.36	(0.49
	6	388.6	82.3	306.3	14.3	19.1	40.1	0.29	0.34	0.25	0.05	0.19	1.98		1.00
	Malt 8	372.0	77.8	294.2	21.7	28.3	39.1	0.25	0.34	0.25	0.05	0.18	1.92		0.98
	11	356.0	76.2	279.8	25.3	40,7	43.5	0.23	0.33	0.30	0.09	0.20	0.06	1.90	0.95
Abed 570 (Imperial Type) 2-rowed	Steep 0	418.5	387.7	30.8	—	—	23.7	0.23	0.10	0.10	0.00	0.00	0.03	0.36	0.18
	6	385.9	81.5	304.4	9.9	22.7	37.4	0.29	0.33	0.21	0.05	0.19	1.62		0.79
	Malt 8	375.9	78.9	297.0	13.2	29.4	35.9	0.32	0.32	0.23	0.02	0.16	1.58		0.75
	11	383.4	79.8	303.6	13.1	22.0	36.8	0.30	0.40	0.19	0.00	0.23	0.05	1.48	0.73
Analytical error		2.1		2.6	2.4		3.3	0.07							

stofte. A.

n	Q = n÷N	N	P	T	P+T		α	Ds	Cv	Pa	Ta	Aml	Rn	Ps	Ex	R′	
								Numerical value of . . . process									
								(1)	(2)	(3)	(4)	(5)	(6)				
Total of nitrogen	insoluble compounds	soluble decomposition products	peptic	tryptic	proteolytic	pH	10 000 steeped corns contain — g. nitrogen in form of Albumin II	Proteid solution:	Albumin conversion:	Peptic action	Tryptic action	Ammonia liberation	Root formation	Peptic action	% Extract of dry malt stuff	g. rootlets for 100 g. malt grains	hussars
.78	7.16	0.62	0.17	0.10	0.27	5.8	0.08	—	—	—	—	—	—	—	5.25	—	
15	4.19	2.96	0.61	1.74	2.35	6.0		2.97	2.85	2.71	2.27	—	0.63	0.70	78.83	3.2	some few
91	3.83	3.08	0.59	1.89	2.48	6.0		3.33	3.08	3.08	2.66	0.90	0 87	0.78	79.60	4 8	many
53	3.48	3.05	0.63	1.91	2.54	6.0		3.68	3.54	3.52	3.06	—	1.25	0.78	78.94	5.6	extraordinarily numerous
45	6.70	0.75	0.15	0.30	0.45	6.0	0.03	—	—	—	—	—	—	—	7.39	—	
73	4.46	2.27	0.38	1.33	1.71	6.0		2.24	2.00	2 46	2.23	—	0.72	0.57	78.56	3.1	none
53	4.07	2.46	0.43	1.51	1.94	6.0		2.63	2.37	2.93	2.65	—	0.92	0.57	78.42	3.9	some few
52	4.01	2.51	0.30	1.55	1.85	6.0		2.69	2.25	2.31	2.16	—	0.93	0.61	78.45	4.7	some few
86	6.96	0.90	0.13	0.39	0.52	6.0	0.10	—	—	—	—	—	—	—	6.80	—	
35	4.25	3.10	0.49	1.98	2.47	6.0		2.71	2.47	2.46	2.10	—	0.51	(0.51)	78.83	3.7	some few
88	3.89	2.99	0.48	1.92	2.40	6.0		3.07	2.83	2.86	2.51	—	0.98	(0.48)	79.06	5.8	several
79	3.68	3.11	0.59	1.96	2.55	5.9		3.28	3.05	3.10	2.64	1.10	1.07	(0.46)	78.60	7.0	extraordinarily numerous
89	7.07	0.82	0.10	0.39	0.49	6.1	0.10	—	—	—	—	—	—	—	7.35	—	
17	4.48	2.69	0.45	1.62	2.07	6.2		2.59	2.36	2.30	1.95	—	0.72	0.61	78.88	2.6	
92	4.29	2.63	0.41	1.58	1.99	6.2		2.78	2.56	2.47	2.16	—	0.97	0.57	78.99	3.5	some few
28	4.63	2.65	0.42	1.53	1.95	6.2		2.44	2.14	2.07	1.75	0.63	0.61	0.55	79.17	3.4	some few
13	0.12	0.05		0.07		0.1					0.07						

TABLE XV. **Year 1913. Barley from Tystofte. A. For 1000 g. dry matter:**

Columns marked			R_1	Gx_1	S_1	n_1	N_1	A_{I_1}	A_{II_1}	P_1	T_1	P_1+T_1	O_1
Sort		After conclusion of normal steeping, germinat. at 14·16° in — days	g. rootlets	g. extract	Acid 2 cc. n. NaOH	g. nitrogen in form of: total of nitrogen	soluble compounds	Albumin I	Albumin II	decomposition products: peptic	decomposition products: tryptic	decomposition products: proteolytic	peptide-bound nitrogen
Tystofte Prentice, 2-rowed	Steep	0	—	51.9	60.7	17.42	1.39	0.60	0.18	0.38	0.22	0.60	0.54
	Malt	8	30.2	727.9	93.8	16.01	6.63	0.92	0.45	1.37	3.90	5.27	2.10
		10	43.0	712.4	97.2	15.47	6.90	0.60	0.74	1.32	4.23	5.55	2.28
		13	49.5	690.5	99.0	14.62	6.83	0.65	0.49	1.41	4.28	5.69	2.28
Svalöf Golden barley 2-rowed	Steep	0	—	71.7	44.2	17.81	1.79	0.79	÷ 0.07	0.36	0.72	1.08	0.43
	Malt	8	28.7	706.5	79.9	16.09	5.43	0.84	0.50	0.91	3.18	4.09	1.79
		10	34.9	697.7	77.0	15.61	5.88	0.69	0.55	1.03	3.61	4.64	1.84
		13	41.4	689.6	77.5	15.59	6.00	0.60	0.98	0.72	3.71	4.43	1.89
Binder barley (Abed 411), 2-rowed	Steep	0	—	65.6	46.2	17.98	2.06	0.64	0.23	0.30	0.89	1.19	1.12
	Malt	6	32.7	700.5	91.7	16.81	7.09	0.66	0.78	1.12	4.53	5.65	2.29
		8	49.6	672.8	89.4	15.73	6.84	0.57	0.78	1 10	4.39	5.49	2.24
		11	57.9	639.9	99.5	15.53	7.11	0.53	0.75	1.35	4.48	5.83	2.17
Abed 570, 2-rowed	Steep	0	—	71.6	55.1	18.34	1.91	0.53	0.23	0.23	0.91	1.14	0.42
	Malt	6	23.0	707.7	87.0	16.67	6.25	0.67	0.77	1.05	3.77	4.82	1.84
		8	30.7	690.5	83.5	16.09	6.11	0.74	0.74	0.95	3.67	4.62	1.74
		11	30.5	705.9	85.6	16.93	6.16	0.70	0.93	0.98	3.56	4.54	1.70
Analytical error			6.4	6.9	8.7	0.34	0.13			0.19			

Found for value		Sort	a	b	m	c	p	s	q	k	U	h	e	f				g	Pʜ·	10 000 barley corns contain g. dry matter	% dry matter in the barley corns	Normal steeping time of the sample in hours
			1000 wet corns weigh gr.	% dry matter in the corns	gr. hydrous matter	♂ cc. $n/_{10}$ acid containing nitrogen	(Quantity of rootlet) gr. rootlets	Root with % dry matter	g. malt grain Corn	% extract (Ball.)	♂ no. of corns 25 cc. dilute wort	acid ♂ cc. $n/_{10}$ NaOH	25 cc. dilute wort contains nitrogen ♂ cc. $n/_{10}$ acid as total nitrogen	precipitable by $SnCl_2$	$HgCl_2$	$FeAc_3$	$UrAc_2$	$MgSO_4$	formol-titratable	50 cc. dilute wort contains nitrogen in form of ammonia ♂ cc. $n/_{10}$ acid			
Tystofte Prentice 2-rowed	Steep	0	42.19	93.90	4.2200	40.5	—	—	—	0.81	59.3	1.3	2.8	0.9	1.6	1.6	1.7	1.3	0.7	0.1	5.9			
		6	39.80	91.97	4.0553	36.5	5.0301	92.41	138.8	8.44	62.8	2.3	10.8	0.8	3.0	3.7	4.0	2.4	3.6	—	6.1	397.8	87.05	48
	Malt	8	39.20	92.40	4.2758	40.0	6.1164	93.15	132.5	8.50	63.8	2.3	11.0	0.8	3.0	3.7	4.2	2.4	3.6	—	6.1			
		11	37.70	92.99	4.1823	38.5	7.8877	92.43	132.4	8.52	66.3	2.5	11.0	0.9	3.1	3.9	4.1	2.3	3.6	0.3	6.1			
Svalöf 2-rowed	Steep	0	39.98	93.41	3.9550	42.0	—	—	—	0.70	62.5	0.7	2.5	0.8	1.2	1.5	1.3	1.0	0.7	0.1	5.9			
		6	38.50	92.75	3.9463	40.0	3.5804	91.95	129.2	8.35	64.9	1.7	9.9	0.8	3 1	3.2	3.8	2.5	3.3	—	5.9	384.9	85.03	72
	Malt	8	37.71	91.64	4.0162	40.5	5.2100	92.09	128.9	8.29	66.3	1.7	10.0	0.8	2.9	3.2	3.6	2.3	3.3	—	5.9			
		11	35.71	93.22	3.9390	40.5	6.8320	91.70	121.5	8.42	70.0	1.9	10.9	0.7	3.1	3.2	3.7	2.4	3.4	0.3	5.9			
Gold. barley (Abed 411) 2-rowed	Steep	0	43.14	90.57	3.7688	38.0	—	—	—	0.67	64.0	0.6	2.6	1.0	1.3	1.6	1.5	1.1	0.8	—	6.0			
		6	40.04	92.33	3.9402	40.0	3.7757	91.77	121.8	8.33	67.6	1.7	11.1	0.9	3.1	3.3	3.6	2.4	4.3	—	6.0	397.6	84.80	72
	Malt	8	38.51	91.32	4.0894	41 0	5.9800	91.10	114.9	8.39	70.8	1.9	12.0	1.0	3.2	3.6	3.7	2.4	4.4	—	6.0			
		11	37.05	89.05	4.1133	41.0	10.4882	90.83	146.2	8.21	75.8	2.2	12.4	0.9	3.2	3.9	4.0	2.2	4.3	0.4	6.0			
Abed 570 Binder barley (Imp.Type) 2-rowed	Steep	0	44.66	91.79	4.0548	45.0	—	—	—	0.87	56.0	1.1	3.1	1.1	1.5	1.7	1.7	1.8	0.8	0.0	5.7			
		6	41.71	92.27	4.0832	44.5	4.1028	92.18	132.8	8.47	59.9	2.1	12.8	1.4	3.5	4.0	4.4	2.9	4.3	—	5.9	422.2	84.78	48
	Malt	8	40.78	92.90	4.0408	43.5	5.2040	92.94	124.6	8.51	61.3	2.2	13.0	1.2	3.5	4.1	4.5	2.9	4.3	0.4	5.9			
		11	39.53	92.84	4.0880	45.5	6.7140	92 71	141.7	8.51	63.2	2.4	13.2	1.2	3.6	4.1	4.6	2.8	4.4	—	5.9			
Tystofte Cross barley 6-rowed	Steep	0	38.60	93.20	4.0337	45.6	—	—	—	0.84	64.8	1.0	3.1	1.2	1.6	1.8	1.9	1.5	0.8	0.1	5.8			
		5	38.11	89.98	4.1905	45.2	2.9196	88.82	136.8	7.97	65.6	2.2	11.1	1.2	3.4	3.3	4.1	2.5	3.7	—	5.9	365.1	87.74	48
	Malt	7	36.58	92.74	4.0410	44.8	4.1285	93.30	127.3	8.36	68.3	2.4	12.9	0.9	3.4	3.6	4.6	2.6	4.2	0.4	5.9			
		9	35.02	92.64	4.2821	48.0	5.4971	94.03	147.3	8.40	71.4	2.5	13.5	1.0	4.5	3.7	4.7	2.6	4.5	—	5.9			
Abed 110 6-rowed	Steep	0	35.06	93.15	4.0535	46.5	—	—	—	0.91	71.3	1.1	3.2	1.1	1.5	1.7	1.7	1.3	1.0	0.1	5.9			
		5	34.39	89.91	4.0973	45.0	3.6532	82.93	124.2	8.15	72.7	2.7	13.7	1.0	3.3	3.9	4.4	2.6	5.0	—	5.9	334.7	88.05	72
	Malt	7	31.51	92.66	3.9650	44.5	4.9015	91.75	119.5	8.42	79.3	2.8	14.5	1.0	3.2	4.3	4.4	2.5	5.2	—	5.9			
		9	31.40	92.51	4.0458	45.0	6.9228	93.63	139.4	8.40	79.6	2.9	14.4	1.1	3.3	4.0	4.7	2.5	5.2	0.4	5.9			

[1]) = days of 24 hours.

TABLE XVII. **Year 1913. Barley fro**

Columns marked			A	Y	Gx	R	I	S	A_I	A_{II}	D	E	F	G	H	O
Sort		After conclusion of normal steeping, germinat. at 14–16° in ... days	10 000 corns gave													
			g. dry matter	g. unaffected matter (husk)	g. extract	g loss as rootlets	g loss by oxidation	acid ∂ cc. n. NaOH	g. nitrogen in form: albumin I	albumin II	peptic decomposition products = P: Denucléin	Propepton	Pepton	tryptic decomposition products = T: Ammonia	Amine. Amide	peptide-bound nitrogen
Tystofte Prentice 2-rowed	Steep	0	396.2	368.4	27.8	—	—	21.9	0.21	0.10	0.07	0.00	0.02	0.01	0.25	0.1
	Malt	6	366.1	69.6	296.5	13.3	16.8	36.6	0.18	0.21	0.28	0.15	0.07	1.52		0.8
		8	362.0	68.1	293.9	16.9	17.3	36.1	0.18	0.20	0.28	0.15	0.11	1.50		0.7
		11	350.6	67.2	283.4	20.8	24.8	37.7	0.19	0.13	0.33	0.17	0 05	0.03	1.42	0.7
Svalöf Golden barley 2-rowed	Steep	0	373.5	350.8	22.7	—	—	11.2	0.18	÷0.03	0.12	0.07	÷0.05	0.01	0.26	0.1
	Malt	6	357.1	73.9	283.2	9.8	6.6	26.2	0.17	0.35	0.15	0.02	0.13	1.32		0.7
		8	345.6	70.1	275 5	14.0	13.9	25.7	0.17	0.25	0.19	0.07	0.08	1.35		0.7
		11	332.9	68.1	264.8	18.4	22.2	27.1	0.14	0.32	0.16	0.02	0.10	0.03	1.40	0.6
Binder barley (Abed 411) 2-rowed	Steep	0	390.8	367.2	23.56	—	—	9.4	0.22	÷0.05	0.11	0.07	÷0.02	0.24		0.1
	Malt	6	369.7	75.9	293.8	11.4	9.7	25.1	0.19	0.27	0.18	0.04	0 07	1.55		0.8
		8	351.6	66.5	285.1	18.3	20.9	26.8	0.20	0.19	0.24	0.08	0.02	1.64		0.8
		11	329.8	61.1	268.7	24.1	36.9	29.0	0.17	0.11	0.31	0.13	0.02	0.04	1.51	0.8
Abed 570 (Imp. Type) 2-rowed	Steep	0	409.9	378.2	31.66	—	—	19.3	0.28	0.12	÷0.02	0.05	0.00	0.00	0.35	0.2
	Malt	6	384.8	73.2	311.6	11.9	13.2	35.6	0.33	0.24	0.25	0.11	0.10	1.96		1.0
		8	378.8	72.4	306.4	15.8	15.3	36.1	0.27	0.25	0.28	0.14	0.09	0.04	1.90	0.9
		11	367.0	70.1	296.9	17.4	25.5	37.5	0.27	0.24	0.29	0.11	0.11	1.90		0.9
Tystofte Cross barley 6-rowed	Steep	0	359.7	333.3	26.4	—	—	15.4	0.26	0.02	0.07	0.04	0.02	0.01	0.25	0.1
	Malt	5	342.9	75.6	267.3	7.2	9.6	33.5	0.26	0.30	0.17	÷0.03	0.18	1.49		0.7
		7	339.3	69.8	269.5	11.1	9.3	35.1	0.18	0.31	0.21	0.04	0.20	0.04	1.66	0.8
		9	324.4	65.2	259.2	12.3	23.0	35.0	0.20	0.27	0.21	0.04	0.19	1.73		0.8
Abed 110 6-rowed	Steep	0	326.6	293.9	32.7	—	—	15.4	0.22	0.00	0.07	0.04	0.00	0.01	0.29	0.2
	Malt	5	309.2	62.0	247.2	9.7	7.7	37.2	0.19	0.20	0.25	0.11	0.10	1.79		0.9
		7	292.0	58.0	234.0	11.9	22.7	35.3	0.18	0.06	0.32	0.20	0.02	1.78		0.9
		9	290.5	57.9	232.6	14.6	21.5	36.4	0.19	0.13	0.26	0.12	0,13	0.04	1.66	0.9
Analytical error			2.1		2.6	2.4		3.3	0.07							

ystofte (marked F).

n	$\frac{Q=}{n \div N}$	N	P	T	P+T		α	Ds	Cv	Pa	Ta	Aml	Rn	Ps	Ex	R'	
								Numerical value of . . . process									
								(1)	(2)	(3)	(4)	(5)	(6)				
total of nitrogen	insoluble compounds	soluble	decomposition products peptic	decomposition products tryptic	decomposition products proteolytic	pH	10000 steeped corns contain — g. nitrogen in form of Albumin II	Proteid solution	Albumin conversion	Peptic action	Tryptic action	Ammonia liberation	Root formation	Peptic action	% Extract of dry malt matter	g. rootlets for 100 g. malt grain	hussars
5.67	5.01	0.66	0.09	0.26	0.35	5.9	0.10	—	—	—	—	—	—	—	7.01	—	
5.16	2.75	2.41	0.50	1.52	2.02	6.1	—	2.26	2.15	2.18	1.77	—	0.51	0.63	80.98	3.6	
5.14	2.72	2.42	0.54	1.50	2.04	6.1	—	2.29	2.19	2.22	1.77	—	0.53	0.62	81.19	4.6	some few
4.86	2.54	2.32	0.55	1.45	2.00	6.1	—	2.47	2.44	2.46	2.00	0.83	0.81	0.59	80.85	6.0	some few
5.95	5.39	0.56	0.14	0.27	0.41	5.9	÷0.03	—	—	—	—	—	—	—	6.09	—	
5.47	3.33	2.14	0.30	1.32	1.62	5.9	—	2.06	1.68	1.69	1.53	—	0.48	0.55	79.31	2.8	
5.33	3.22	2.11	0.34	1.35	1.69	5.9	—	2.17	1.89	1.90	1.70	—	0.62	0.54	79.72	4.0	some few
5.14	2.97	2.17	0.28	1.43	1.71	5.9	—	2.42	2.07	2.11	1.79	0.83	0.81	0.52	79.56	5.6	
6.09	5.52	0.57	0.16	0.24	0.40	6.0	÷0.05	—	—	—	—	—	—	—	6.03	—	
5.69	3.39	2.30	0.29	1.55	1.84	6.0	—	2.13	1.81	1.84	1.71	—	0.40	0.71	79.49	3.1	
5.41	3.04	2.37	0.34	1.64	1.98	6.0	—	2.48	2.24	2.26	2.08	—	0.68	0.71	79.43	5.2	some
5.17	2.88	2.29	0.46	1.55	2.01	6.0	—	2.64	2.48	2.53	2.23	—	0.92	0.63	81.46	7.2	many
6.94	6.16	0.78	0.03	0.35	0.38	5.7	0.12	—	—	—	—	—	—	—	7.72	—	
6.37	3.38	2.99	0.46	1.96	2.42	5.9	—	2.78	2.66	2.73	2.30	—	0.57	0.81	80.98	3.1	
6.15	3.18	2.97	0.51	1.94	2.45	5.9	—	2.98	2.85	2.99	2.51	0.83	0.79	0.78	80.85	4.2	some few
6.16	3.24	2.92	0.51	1.90	2.41	5.9	—	2.92	2.80	2.93	2.45	—	0.78	0.77	80.89	4.7	some few
6.11	5.44	0.67	0.13	0.26	0.39	5.8	0.02	—	—	—	—	—	—	—	7.34	—	
6.75	3.38	2.37	0.32	1.49	1.81	5.9	—	2.06	1.78	1.78	1.59	—	0.36	0.62	73.38	2.1	
6.68	3.04	2.64	0.45	1.70	2.15	5.9	—	2.40	2.11	2.19	1.87	0.46	0.43	0.69	79.43	3.2	none
6.50	2.85	2.65	0.44	1.73	2.17	5.9	—	2.59	2.34	2.40	2.09	—	0.61	0.71	79.93	3.7	
6.63	5.00	0.63	0.11	0.30	0.41	5.9	0.00	—	—	—	—	—	—	—	7.95	—	
6.29	2.65	2.64	0.46	1.79	2.25	5.9	—	2.35	2.15	2.18	1.83	—	0.34	0.76	79.95	2.9	none
4.95	2.39	2.56	0.54	1.78	2.32	5.9	—	2.61	2.55	2.59	2.16	—	0.68	0.72	80.11	4.1	none
4.89	2.36	2.53	0.51	1.70	2.21	5.9	—	2.64	2.51	2.54	2.14	0.77	0.74	0.71	80.05	5.0	some few
0.13	0.12	0.05	0.07			0.1		0.07									

TABLE XVIII. **Year 1913. Barley from Tystofte. F. For 1000 g. dry matter:**

Columns marked			R_1	Gx_1	S_1	n_1	N_1	A_{I_1}	A_{II_1}	P_1	T_1	P_1+T_1	O_1
Sort		After conclusion of normal steeping, germinat. at 14-16° in — days	g. rootlets	g. extract	acid ≙ cc. n. NaOH	g. nitrogen in form of: total of nitrogen	soluble compounds	albumin: I	albumin: II	decomposition products: peptic	decomposition products: tryptic	decomposition products: proteolytic	peptide-bound nitrogen
Tystofte Prentice 2-rowed	Steep	0	—	69.9	55.1	14.25	1.66	0.53	0.25	0.23	0.65	0.88	0.43
	Malt	8	33.4	745.4	92.0	12.97	6.06	0.45	0.53	1.26	3.82	5.08	2.01
		10	42.5	738.9	90.8	12.92	6.08	0.45	0.50	1.36	3.77	5.13	1.99
		13	52.3	712.5	94.8	12.22	5.83	0.48	0.33	1.38	3.65	5.03	1.91
Svalöf Golden barley 2-rowed	Steep	0	—	59.0	29.1	15.46	1.45	0.47	÷ 0.08	0.36	0.70	1.06	0.42
	Malt	8	25.5	735.8	68.1	14.21	5.56	0.44	0.91	0.78	3.43	4.21	1.84
		10	36.4	715.7	66.8	13.85	5.48	0.44	0.65	0.88	3.51	4.39	1.82
		13	47.8	688.0	70.4	13.35	5.64	0.36	0.83	0.73	3.72	4.45	1.77
Binder barley (Abed 411) 2-rowed	Steep	0	—	59.4	23.6	15.32	1.43	0.55	÷ 0.13	0.40	0.60	1.00	0.43
	Malt	8	28.7	738.9	63.1	14.31	5.78	0.48	0.68	0.73	3.90	4.63	2.21
		10	46.0	717.0	67.4	13.61	5.96	0.50	0.48	0.86	4.12	4.98	2.21
		13	60.6	675.8	72.9	13.00	5.76	0.43	0.28	1.16	3 90	5.06	2.01
Abed 570 2-rowed	Steep	0	—	75.1	45.7	16.44	1.85	0.66	0.28	0.07	0.83	0.90	0.47
	Malt	8	28.2	738.2	84.3	15.09	7.08	0.78	0.57	1.09	4.64	5.73	2.39
		10	37.4	725.9	85.5	14.57	7.04	0.64	0.59	1.21	4.60	5.81	2.32
		13	41.2	703.4	88.8	14.59	6.92	0.64	0.57	1.21	4.50	5.71	2.30
Tystofte Cross barley 6-rowed	Steep	0	—	72.3	42.2	16.74	1.84	0.71	0.05	0.36	0.71	1.07	0.47
	Malt	6	19.7	732.1	91.8	15.75	6.49	0.71	0.82	0.88	4.08	4.96	2.16
		8	30.4	738.3	96.1	15.56	7.23	0.49	0.85	1.23	4.66	5.89	2.36
		11	33.7	709.9	95.9	15.06	7.26	0.55	0.74	1.21	4.74	5.95	2.41
Abed 110 6-rowed	Steep	0	—	97.7	46.0	16.82	1.88	0.66	0.00	0.33	0.90	1.23	0.60
	Malt	6	29.0	738.6	111.2	15.81	7.89	0.57	0.60	1.37	5.35	6.72	2.87
		8	35.6	699.2	105.5	14.79	7.65	0.54	0.18	1.61	5.32	6.93	2.75
		11	43.6	695.0	108.8	14.61	7.56	0.57	0.39	1.52	5.08	6.60	2.72
Analytical error c.			6.4	6.9	8.7	0.34	0.13	0.19					

TABLE XIX. **Year 1913. Barley from Abed.**

Found for value			a	b	m	c	p	s	q	k	U	h	e	f						g	p_H			
Sort		After conclusion of normal steeping germinated at 14—16° in . . . days[1])	1000 wet corns weigh g.	% dry matter in the corns	gr. hydrous matter	containing nitrogen ≈ cc. n/10 acid	Quantity of rootlet: Root: g. rootlets	Quantity of rootlet: Root: with % dry matter	Quantity of rootlet: Corn: g. malt grains	% extract (Ball.)	25 cc. dilute wort ≈ no. of corns	25 cc. dilute wort contains: acid ≈ cc. n/10 NaOH	25 cc. dilute wort contains: total nitrogen	25 cc. dilute wort contains: nitrogen ≈ cc. n/10 acid as precipitable by $SnCl_2$	$HgCl_2$	$FeAc_3$	$UrAc_2$	$MgSO_4$	formol-titratable	50 cc. dilute wort contains nitrogen in form of ammonia ≈ cc. n/10 acid		10 000 barley corns contain g. dry matter	% dry matter in the barley corns	Normal steeping time of the sample in hours
Tystofte Prentice 2-rowed	Steep	0	42.12	93.46	4.0743	36.6	—	—	—	0.89	59.3	1.5	2.8	0.6	1.5	1.6	1.6	1.2	0.7	0.1	5.8			
	Malt	8	37.51	92.71	3.8923	32.1	6.9275	89.83	114.1	8.59	66.6	2.8	11.8	0.8	2.8	3.2	4.1	1.8	4.4	—	6.0	402.4	87.86	48
		10	36.04	93.23	3.9437	32.3	9.4747	87.81	108.1	8.59	69.4	2.9	12.0	0.7	2.5	3.6	3.7	1.6	4.4	0.6	6.0			
		13	34.97	92.66	3.7273	31.5	10.4634	88.26	102.9	8.55	71.5	3.1	12.0	0.7	2.5	3.5	3.7	1.5	4.4	0.7	6.0			
Svalöf Gold. barley 2-rowed	Steep	0	40.60	92.93	4.0786	37.3	—	—	—	0.86	61.6	1.3	2.8	0.8	1.4	1.5	1.6	1.1	0.7	0.1	6.0			
	Malt	8	39.09	92.60	4.0816	36.4	3.8112	91.03	139.0	8.54	64.0	2.2	9.6	0.9	2.7	2.9	3.6	2.0	3.0	—	6.0	392.7	88.78	24
		10	37.24	93.12	4.0300	35.6	4.3532	93.32	119.5	8.56	67.1	2.2	9.5	0.9	2.7	2.8	3.6	2.1	2.9	—	6.0			
		13	36.62	93.67	4.0480	36.8	4.5906	92.89	118.4	8.60	68.3	2.3	9.6	0.8	2.7	2.9	3.7	2.1	2.9	—	6.0			
Binder barley (Abed 411) 2-rowed	Steep	0	43.38	91.65	4.0062	36.1	—	—	—	0.74	57.6	1.2	2.8	0.9	1.4	1.5	1.6	1.3	0.6	—	5.9			
	Malt	8	39.58	90.78	4.0000	34.6	6.6310	87.95	120.8	8.43	63.2	2.6	11.6	1.1	2.9	3.8	4.0	2.2	4.2	0.3	6.0	412.2	88.30	48
		10	38.18	92.45	4.0650	35.6	7.8370	87.55	115.8	8.60	65.5	2.9	12.3	1.0	2.8	3.6	4.2	2.1	4.2	—	6.0			
		13	36.63	91.25	4.2457	37.5	7.2083	87.44	93.1	8.45	68.2	3.0	12.3	1.1	2.9	3.8	4.2	2.1	4.2	0.4	6.0			
Abed 570 2-rowed	Steep	0	45.60	91.62	4.0612	37.5	—	—	—	0.90	54.8	1.5	2.8	0.9	1.4	1.5	1.7	1.2	0.6	0.1	5.8			
	Malt	8	42.00	92.83	4.1110	37.9	4.1873	90.96	133.5	8.67	59.5	2.9	12.0	0.9	3.0	4.0	4.4	2.3	4.0	—	5.9	419.1	87.21	24
		10	41.06	92.91	4.0050	37.2	4.7303	93.53	131.5	8.67	60.9	2.9	11.9	0.8	3.0	3.8	4.4	2.3	3.9	—	5.9			
		13	40.19	93.15	4.0379	38.2	6.0290	93.46	139.4	8.77	62.2	3.1	12.3	0.9	3.0	3.6	4.5	2.2	4.0	0.4	5.9			
Abed 110 6-rowed	Steep	0	31.01	93.61	4.0113	37.2	—	—	—	0.86	80.6	1.5	2.6	0.6	1.1	1.4	1.5	0.9	0.6	0.1	5.6			
	Malt	6	30.37	90.34	4.0063	36.2	3.9101	89.82	122.4	8.19	82.3	2.8	12.1	0.8	2.6	2.8	3.9	1.6	4.4	—	5.9	301.8	88.81	48
		8	26.83	92.13	4.0792	37.1	5.9456	91.99	118.4	8.33	93.2	3.1	13.1	0.7	2.5	2.8	4.1	1.3	4.8	—	5.9			
		11	27.41	90.38	4.1173	37.9	6.5106	92.01	133.3	8.09	91.2	3.3	13.5	0.7	2.5	2.9	4.2	1.3	4.8	0.5	5.9			

[1]) = days of 24 hours.

TABLE XX. **Year 191**

Columns marked			A	Y	Gx	R	I	S	A_I	A_{II}	D	E	F	G	H	O
Sort		After conclusion of normal steeping, germinated at 14—16° in . . . days	10 000 corns gave													
			g. Dry matter	g. Unaffected matter (husk)	g. extract	g. loss as rootlets	g. loss by oxidation	acid ≙ cc. n. NaOH	g. nitrogen in form: albumin I	albumin II	peptic decomposition products = P: Deuteroalbumin	Propepton	Pepton	tryptic decomposition products = T: Ammonia	Amine-Amide	peptide-bound nitrogen
Tystofte Prentice 2-rowed	Steep	0	393.7	363.2	30.5	—	—	25.3	0.14	0.11	0.10	0.03	0.00	0.01	0.27	0.17
	Malt	8	347.8	63.2	284.6	20.5	25.4	42.0	0.17	0.13	0.29	0.08	0.19	1.62		0.92
		10	335.9	62.8	273.1	27.7	30.1	41.8	0.14	÷0.05	0.41	0.23	0.02	0.06	1.61	0.89
		13	324.1	60.1	264.0	31.4	38.2	43.4	0.14	÷0.05	0.40	0.20	0.03	0.07	1.56	0.86
Svalöf Golden barley 2-rowed	Steep	0	377.1	348.7	28.4	—	—	21.1	0.18	0.05	0.09	0.02	0.02	0.01	0.27	0.16
	Malt	8	362.0	67.2	294.8	9.8	5.3	34.4	0.20	0.19	0.20	0.05	0.15	1.21		0.66
		10	346.8	65.5	281.3	12.7	17.6	32.8	0.19	0.23	0.14	0.02	0.17	1.23		0.60
		13	343.0	65.2	277.8	13.2	20.9	33.7	0.16	0.23	0.16	0.04	0.17	1.21		0.59
Binder barley (Abed 411) 2-rowed	Steep	0	397.6	371.4	26.2	—	—	20.8	0.22	0.08	0.04	0.02	0.03	0.29		0.15
	Malt	8	359.3	64.5	294.8	19.1	19.2	41.2	0.24	0.05	0.35	0.20	0.05	0.03	1.65	0.93
		10	352.9	62.7	290.2	22.6	22.1	44.3	0.21	0.07	0.32	0.17	0.13	1.73		0.90
		13	334.4	61.0	273.4	24.8	38.4	44.0	0.23	0.02	0.35	0.18	0.08	0.04	1.62	0.86
Abed 570 2-rowed	Steep	0	417.8	384.3	33.5	—	—	27.4	0.23	0.06	0.07	0.02	0.05	0.01	0.28	0.15
	Malt	8	390.0	68.1	321.9	12.0	15.8	48.7	0.21	0.10	0.40	0.23	0.09	1.79		0.94
		10	381.4	66.8	314.6	13.8	22.6	47.6	0.18	0.17	0.34	0.18	0.14	1.73		0.90
		13	374.4	62.8	311 6	16.3	27.1	49.9	0.20	0.17	0.31	0.13	0.20	0.05	1.71	0.90
Abed 110 6-rowed	Steep	0	290.2	268.5	21.7	—	—	18.6	0.10	0.01	0.08	0.05	0.02	0.01	0.18	0.10
	Malt	6	274.4	55.0	219.4	8.7	7.1	34.0	0.14	0.09	0.21	0.04	0.18	1.40		0.75
		8	247.2	50.2	197.0	12.4	30.6	33.3	0.11	0.05	0.22	0.04	0.20	1.35		0.72
		11	247.7	52.4	195.3	12.3	30.2	36.2	0.11	0.02	0.25	0.07	0.19	0.04	1.39	0.74
Analytical error			2.1		2.6	2.4		3.3	0.07							

rley from Abed.

n	Q = n÷N	N	P	T	P+T	p_H	α	Ds	Cv	Pa	Ta	Aml	Rn	Ps	Ex	R′	
								Numerical value of . . . process									
								(1)	(2)	(3)	(4)	(5)	(6)				
Total of nitrogen	insoluble compounds	soluble	decomposition products: peptic	tryptic	proteolytic		10 000 steeped corns contain — g. nitrogen in form of Albumin II	Proteid solution:	Albumin conversion:	Peptic action	Tryptic action	Ammonia liberation	Root formation	Peptic action	% Extract of dry malt matter	g. rootlets for 100 g. malt grains	hussars
30	4.64	0.66	0.13	0.28	0.41	5.8	0.11	—	—	—	—	—	—	—	7.75	—	
33	1.85	2.48	0.56	1.62	2.18	6.0		2.79	2.77	2.74	2.31	—	0.97	0.75	81.85	6.1	extra-ordinarily numerous
13	1.71	2.42	0.66	1.67	2.33	6.0		2.93	3.09	3.09	2.56	1.22	1.17	0.72	81.32	8.8	
14	1.79	2.35	0.63	1.63	2.26	6.0		2.85	3.01	3.01	2.51	1.22	1.16	0.69	81.47	10.2	
20	4.56	0.64	0.13	0.28	0.41	6.0	0.05	—	—	—	—	—	—	—	7.53	—	
88	2.88	2.00	0.40	1.21	1.61	6.0		1.68	1.54	1.52	1.25	—	0.32	0.50	81.44	2.7	few
61	2.63	1.98	0.33	1.23	1.56	6.0		1.93	1.75	1.74	1.54	—	0.59	0.44	81.12	3.6	few
66	2.69	1.97	0.37	1.21	1.58	6.0		1.87	1.69	1.71	1.47	—	0.54	0.43	81.00	3.9	some
47	4.79	0.68	0.09	0.29	0.38	5.9	0.08	—	—	—	—	—	—	—	6.58	—	
79	2.22	2.57	0.60	1.68	2.28	6.0		2.57	2.60	2.58	2.07	—	0.68	0.78	82.05	5.5	many
68	2.05	2.63	0.62	1.73	2.35	6.0		2.74	2.75	2.76	2.23	—	0.79	0.75	82.21	6.8	
53	2.01	2.52	0.61	1.66	2.27	6.0		2.78	2.84	2.83	2.31	—	0.94	0.71	81.77	7.7	
90	5.18	0.72	0.14	0.29	0.43	5.8	0.06	—	—	—	—	—	—	—	8.01	—	
42	2.60	2.82	0.72	1.79	2.51	5.9		2.58	2.54	2.56	1.98	—	0.48	0.79	82.55	3.1	some few
34	2.60	2.74	0.66	1.73	2.39	5.9		2.58	2.47	2.52	2.00	—	0.56	0.75	82.49	3.6	
32	2.55	2.77	0.64	1.76	2.40	5.9		2.63	2.52	2.55	2.05	0.62	0.58	0.75	83.25	4.3	
03	3.58	0.45	0.15	0.19	0.34	5.6	0.01	—	—	—	—	—	—	—	7.48	—	
84	1.78	2.06	0.43	1.40	1.83	5.9		1.80	1.72	1.68	1.40	—	0.19	0.65	79.95	3.2	some
42	1.45	1.97	0.46	1.35	1.81	5.9		2.13	2.09	2.08	1.77	—	0.61	0.62	79.67	5.0	a good few
53	1.46	2.07	0.51	1.43	1.94	5.9		2.12	2.11	2.10	1.74	0.53	0.50	0.64	78.81	4.9	a good few
13	0.12	0.05	0.07			0.1		0.07									

TABLE XXI. **Year 1913. Barley from Abed. For 1000 g. dry matter:**

Columns marked			R_1	Gx_1	S_1	n_1	N_1	AI_1	AII_1	P_1	T_1	P_1+T_1	O_1
Sort		After conclusion of normal steeping, germinat. at 14-16° in — days	g. rootlets	g. extract	acid ∾ cc. n NaOH	g. nitrogen in form of: total of nitrogen	soluble compounds	albumin: I	albumin: II	decomposition products: peptic	decomposition products: tryptic	decomposition products: proteolytic	peptide-bound nitrogen
Tystofte Prentice, 2-rowed	Steep	0	—	75.8	62.9	13.17	1.64	0.35	0.27	0.32	0.70	1.02	0.42
	Malt	8	50.9	707.2	104.4	10.76	6.16	0.42	0.32	1.39	4.03	5.42	2.29
		10	68.8	678.7	103.9	10.26	6.01	0.35	÷ 0.12	1.64	4.15	5.79	2.21
		13	78.0	656.0	107.8	10.29	5.84	0.35	÷ 0.12	1.57	4.05	5.62	2.14
Svalöf Golden barley 2-rowed	Steep	0	—	72.3	53.7	13.24	1.63	0.46	0.13	0.33	0.71	1.04	0.41
	Malt	8	25.0	750.6	87.6	12.42	5.09	0.51	0.48	1.02	3.08	4.10	1.68
		10	32.3	716.2	83.5	11.74	5.04	0.48	0.59	0.84	3.13	3.97	1.53
		13	33.6	707.3	85.8	11.86	5.02	0.41	0.59	0.94	3.08	4.02	1.50
Binder barley (Abed 411). 2-rowed	Steep	0	—	63.6	50.5	13 27	1.65	0.53	0.19	0.22	0.70	0 92	0.36
	Malt	8	46.3	715.2	100.0	11.62	6.23	0.58	0.12	1.46	4.08	5.54	2.26
		10	54.8	704.0	107.5	11.35	6.38	0.51	0.17	1.50	4.20	5.70	2.18
		13	60.2	663.3	106.7	10.99	6.11	0.56	0.05	1.48	4.03	5.51	2.09
Abed 570, 2-rowed	Steep	0	—	79.9	65.4	14.08	1.72	0.55	0.14	0.33	0.69	1.02	0.36
	Malt	8	28.6	768.1	116.2	12.93	6.73	0.50	0.24	1.72	4.27	5.99	2.24
		10	32.9	750.6	113.6	12.74	6.54	0.43	0.41	1.57	4.13	5.70	2.15
		13	38.9	743.5	119.1	12.69	6.61	0.48	0.41	1.53	4.20	5.73	2.15
Abed 110, 6-rowed	Steep	0	—	71.9	61.6	13.35	1.49	0.33	0.03	0.50	0.63	1.13	0.33
	Malt	6	28.8	726.9	112.6	12.72	6.82	0.46	0.30	1.42	4.64	6.06	2.48
		8	41.1	652.7	110.3	11.33	6.53	0.36	0.17	1.52	4.47	5.99	2.39
		11	40.7	647.0	119.9	11.69	6.86	0.36	0.07	1.69	4.74	6.43	2.45
Analytical error c.			6.4	6.9	8.7	0.34	0.13	0.19					

barley grown at Tystofte in 1914, there was a great difference observable between Nos. 1 and 2 on the one hand, and No. 3 on the other. The quantity of soluble nitrogen compounds likewise varied greatly from year to year for the same sort, and appears to be in some degree dependent upon the total amount of nitrogen in the grain; at any rate, in most of the races.

The albumin conversion process was here, as in the case of the Swedish samples, mainly unsatisfactory. The best results were those obtained with the samples from Abed. where Binder barley and Abed 110, both for 1913 and 1914, turned out well, and the three other sorts showed, — at least for the one year — a more or less complete conversion of albumin.

The ratio between peptase action (3) and the proteïd solution process (1) was satisfactory in practically all cases, (3) being very nearly equal to (1) and in some instances even slightly greater.

As shown in the case of the Svalöf samples of Golden barley, so also the Danish samples tend to indicate this sort as comparatively poor in proteolytic enzymes, even great variations of the total amount of nitrogenous compounds being accompanied by almost equal amounts of formol-titratable nitrogen (O) and of proteolytic decomposition products (P + T). Only the sample from Tystofte 1914 shows very high values for these compounds, but in this case, the malt was damaged by micro-organisms, the wort produced was very dark, and there is thus no doubt that the micro-organisms have occasioned strong decomposition processes. The results for Golden barley agree well with the results arrived at in the Brewery; to wit, that this sort is but little suited for malting purposes.

The samples of Tystofte Prentice, Nos. 1 and 2 from Tystofte 1914, which were grown one with the other without the addition of magnium sulphate to the soil, exhibited practically no difference as regards ash content. 20.00 g. dry barley matter gave:

	SiO_2	CaO	$Mg_2P_2O_7$
No. 1 (with $MgSO_4$)	0.0700 g.	0.0152 g.	0.1197 g.
- 2 (without —)	0.0733 -	0.0144 -	0.1204 -

The soil must thus beforehand have had no lack of magnium salts. Analyses of the wort, again, do not appear to indicate any alteration in the character of the barley owing to addition of magnium sulphate, as the differences found in the composition of the wort fall within the limits of experimental error.

TABLE XXII

Year 1914. Barley from Tystofte.

Found for value		a	b	m	c	p	s	q	k	U	h	e	f						g				
Sort	After conclusion of normal steeping, germinated at 14—16° in . . . days[1])	1000 wet corns weigh gr.	% dry matter in the corns	gr. hydrous matter	containing nitrogen ∞ cc. n/10 acid	Quantity of rootlet: Root g. rootlets	Quantity of rootlet: Root with % dry matter	Quantity of rootlet: Corn g. malt grain	% extract (Ball.)	25 cc. dilute wort ∞ no. of corns	25 cc. dilute wort contains: acid ∞ cm³ n/10 NaOH	25 cc. dilute wort contains: nitrogen ∞ cc. n/10 acid as: total nitrogen	precipitable by: $SnCl_2$	precipitable by: $HgCl_2$	precipitable by: $FeAc_3$	precipitable by: $UrAc_2$	precipitable by: $MgSO_4$	formol-titratable	50 cc. dilute wort contains nitrogen in form of ammonia ∞ cc. n/10 acid	p_H	10 000 barley corns contain g. dry matter	% dry matter in the corns	Normal steeping time of the sample in hours
Tystofte Prentice, 2-rowed without $MgSO_4$ Nr. 2	Steep 0	43.68	91.77	4.0299	47.0	—	—	—	0.80	57.2	1.0	3.2	1.2	1.7	1.6	1.8	1.4	0.9	0.1	5.9			
	Malt 8	36.50	93.28	4.0316	47.0	8.7282	91.48	128.9	8.44	68.5	2.7	13.6	1.3	3.5	4.1	4.7	2.6	5.0	0.5	5.9			
	Malt 10	34.64	93.37	4.0867	47.1	10.4945	91.18	—	8.44	72.2	2.9	14.5	1.3	3.4	3.9	4.8	2.5	5.3	0.6	5.9	398.5	89.39	96
	Malt 13	34.58	93.68	4.0703	47.1	13.6426	92.03	119.2	8.38	72.3	3.1	14.9	1.2	3.5	4.0	4.8	2 4	5.5	0.6	5.9			
Tystofte Prentice, 2-rowed with $MgSO_4$ Nr. 1	Steep 0	42.11	91.70	4.0021	47.0	—	—	—	0.74	59.4	0.9	3.2	1.1	1.7	1.9	1.9	1.4	0.8	0.2	6.0			
	Malt 8	36.46	92.37	4.2310	48.3	8.2405	92.68	111.7	8.40	68.6	2.7	13.7	1.3	3.8	4.4	5.1	2.9	4.7	0.4	5.9			
	Malt 10	35.13	92.04	4.2099	47.9	10.9420	91.32	107.5	8.36	71.2	2.8	14.4	1.3	3.6	4.2	5.0	2.6	4.9	0.5	5.9	397.0	88.72	96
	Malt 13	34.20	91.38	4.0329	46.7	14.8339	90.88	127.3	8.25	73.1	2.9	14.5	1.3	3.7	4.8	5.2	2.6	4.7	0.5	5.9			
Tystofte Prentice, 2-row., Nr. 3	Steep 0	42.26	93.25	4.0023	60.4	—	—	—	0.82	59.2	1.0	3.4	1.0	1.7	2.0	2 1	1.6	0.7	0.1	5.7			
	Malt 8	38.11	89 96	4.0139	59.5	7.4397	90.16	128.6	7.92	65.6	2.5	14.9	1.3	4.3	5.3	5.5	3.5	4.5	0.5	5.8			
	Malt 10	36.16	92.70	4.0201	60.5	8.9925	91.61	116.9	8.18	69.1	2.8	16.3	1.5	4.4	5.4	5.9	3.5	5.0	0.5	5.8	393.2	88.47	72
	Malt 13	34.96	91.81	4.0511	62.5	10.7741	90.69	122.2	8.04	71.5	2.9	17.0	1.4	4.6	5.5	6.0	3.6	5.2	0.5	5.8			

Svalöf Golden barley 2-rowed	Steep	0	35.63	92.82	4.0433	62.5	—	—	—	0.91	70.2	1.0	3.6	1.0	1.5	1.8	1.9	1.3	0.9	0.2	5.9	335.7	88.29	72
	Malt	8	32.49	88.74	4.0074	62.0	5.6436	90.91	119.4	7.88	76.9	3.0	16.2	1.2	4.5	4.7	5.3	3.5	5.5	1.0	5.9			[2]
		10	30.47	90.28	4.0390	63.9	7.6115	90.95	116.4	8.15	82.1	3.3	18.9	1.2	4.6	5.0	6.0	3.5	6.7	1.0	5.9			[2]
		13	29.31	89.4	4.0237	65.8	7.8730	90.71	120.4	8.05	85.3	3.9	21.5	1.1	4.7	5.6	6.3	3.3	9.2	1.4	5.9			
Binder barley (Abed 411), 2-rowed	Steep	0	41.24	92.12	4.0680	64.7	—	—	—	0.91	60.6	1.0	3.8	1.0	1.9	2.0	2.1	1.5	0.9	0.1	5.7	382.0	87.92	48
	Malt	8	37.18	91.61	4.0299	64.5	5.9740	90.71	122.2	8.13	67.2	2.7	17.0	1.1	4.6	5.9	5.6	3.8	5.7	0.6	5.9			
		10	36.54	92.66	4.0015	64.9	6.7232	91.25	117.6	8.22	68.4	2.8	17.7	1.4	4.8	6.0	6.2	3.9	5.9	0.5	5.9			
		13	36.02	89.81	4.0114	64.7	9.4308	90.22	154.2	7.95	69.4	3.0	18.9	1.3	4.9	6.6	6.4	3.7	6.2	0.5	5.8			
Abed 570, 2-rowed	Steep	0	39.64	92.18	4.0557	61.0	—	—	—	0.92	63.1	1.0	3.2	1.0	1.7	1.9	1.8	1.5	0.6	0.1	5.6	366.6	88.39	24
	Malt	8	36.91	91.89	4.0752	61.8	4.9455	91.26	132.5	8.07	67.7	2.1	13.2	1.2	3.9	4.7	4.8	3.3	4.0	—	5.9			
		10	35.38	90.11	4.0105	60.5	5.4430	91.71	126.4	8.27	70.7	2.3	14.3	1.1	4.1	5.4	5.5	3.3	4.4	0.4	5.9			
		13	35.53	92.20	4.0561	62.1	5.4861	91.37	131.2	8.21	70.4	2.6	15.5	1.2	4.4	5.5	5.9	3.6	4.7	0.5	5.9			
Tystofte Cross barley 6-rowed	Steep	0	32.91	93.77	4.0154	58.1	—	—	—	0.94	76.0	1.0	3.1	1.2	1.7	1.8	2.0	1.4	0.7	0.1	5.6	299.3	89.62	24
	Malt	6	31.07	92.84	4.0286	58.0	3.0054	90.30	140.5	8.04	80.5	1.9	13.1	1.5	3.8	4.4	4.4	3.2	4.2	0.4	6.0			
		8	30.22	92.14	4.1384	59.7	3.0127	89.71	145.8	8.01	82.7	2.1	13.5	1.3	4.0	5.0	4.6	3.2	4.2	0.4	5.9			
		11	30.27	92.54	4.0677	59.0	2.9320	91.49	137.9	8.04	82.6	2.1	13.9	1.5	4.2	4.9	4.8	3.3	4.4	0.4	5.9			
Abed 110, 6-rowed	Steep	0	23.53	93.58	4.0381	56.8	—	—	—	1.05	106.3	1.1	3.9	1.3	1.8	2.0	1.8	1.2	1.2	—	5.8	221.9	89.40	48
	Malt	6	22.50	91.95	4.1126	58.5	2.4800	88.28	115.1	7.93	111.1	2.9	16.4	2.0	4.4	4.9	5.5	3.2	6.0	—	5.9			
		8	21.33	91.89	4.0039	57.3	3.9673	89.77	118.0	7.99	117.2	3.5	19.2	2.1	4.3	5.4	5.5	2.8	7.3	—	5.8			
		11	20.80	89.42	4.0961	59.1	5.8250	89.53	145.4	7.79	120.2	4.1	21.0	1.4	4.1	5.0	5.7	2.5	8.3	—	5.8			

[1]) = days of 24 hours. [2]) The malt (in these two samples) was greatly overgrown with micro-organisms at the conclusion of the experiment; the wort produced was very dark.

TABLE XXIII. **Year 19**

Columns marked		A	Y	Gx	R	I	S	A_I	A_{II}	D	E	F	G	H	O
Sort	After conclusion of normal steeping, germinat. at 14 16° in — days	10 000 corns gave													
		g. dry matter	g. unaffected matter (husk)	g. extract	g. loss as rootlets	g. loss by oxidation	acid ∞ c. n. NaOH	g. nitrogen in the form albumin I	albumin II	peptic decomposition products = P Deuteroalbumin	Propepton	Pepton	tryptic decomposition products = T Ammonia	Amine-Amide	peptide-bound nitrogen
Tystofte Prentice, 2-rd. without $MgSO_4$ with $MgSO_4$ Nr. 1 Steep	0	386.1	360.7	25.4	—	—	14.8	0.26	0.04	0.10	0.04	0.02	0.02	0.27	0.1
Malt	8	336.7	66.7	270.0	24.9	24.5	39.1	0.26	0.19	0.32	0.13	0.13	0.04	1.73	0.9
	10	323.3	64.4	258.9	32.6	30.2	39.8	0.26	0.15	0.30	0.11	0.17	0.05	1.80	0.9
	13	312.5	63.9	248.6	36.2	37.4	39.5	0.25	0.07	0.38	0.22	0.08	0.05	1.73	0.8
Tystofte Prentice without $MgSO_4$ Nr. 2 Steep	0	400.8	372.3	28.5	—	—	17.5	0.29	0.06	0.06	÷0.01	0.05	0.01	0.33	0.2
Malt	8	340.4	69.0	271.4	22.6	37.8	38.7	0.27	0.14	0.30	0.13	0.11	0.05	1.79	1.0
	10	323.4	65.9	257.5	—	—	40.3	0.25	0.14	0.28	0.09	0.18	0.06	1.81	1.0
	13	324.0	69.0	255.0	36.4	40.4	42.5	0.23	0.13	0.32	0.10	0.15	0.06	1.89	1.0
Tystofte 2-rd., Nr. 3 Steep	0	394.0	365.8	28.2	—	—	16.7	0.23	0.08	0.10	0.06	0.03	0.01	0.30	0.1
Malt	8	342.8	77.5	265.3	20.0	31.2	38.3	0.26	0.27	0.39	0.21	0.04	0.05	1.96	0.9
	10	335.2	75.1	260.1	25.5	33.3	40.4	0.30	0.22	0.38	0.19	0.11	0.05	2.04	1.0
	13	320.9	74.0	246.9	27.9	45.2	40.6	0.28	0.25	0.38	0.17	0.09	0.05	2.11	1.0
Svalöf Golden barley 2-rd. Steep	0	330.8	304.4	26.4	—	—	13.5	0.19	0.03	0.08	0.05	0.02	0.02	0.32	0.1
Malt	8	288.2	62.9	225.3	14.0	28.6	38.9	0.23	0.37	0.22	0.04	0.11	0.09	1.89	1.0
	10	275.0	56.2	218.8	18.1	37.7	40.6	0.21	0.31	0.26	0.07	0.18	0.09	2.11	1.1
	13	262.1	54.1	208.0	17.4	51.3	45.4	0.18	0.22	0.38	0.14	0.11	0.11	2.40	1.5
Binder barley (Abed 411) 2-rd. Steep	0	379.9	349.3	30.6	—	—	16.0	0.23	0.10	0.11	0.02	0.02	0.01	0.39	0.2
Malt	8	340.6	74.7	265.9	16.5	22.8	39.4	0.23	0.29	0.44	0.27	÷0.06	0.06	2.31	1.1
	10	338.6	74.3	264.3	19.1	22.2	40.5	0.29	0.26	0.43	0.25	0.04	0.05	2.30	1.2
	13	323.4	71.4	252.0	19.9	36.6	43.2	0.26	0.15	0.58	0.34	÷0.04	0.05	2.49	1.2
Abed 570 2-rd. Steep	0	365.4	335.7	29.7	—	—	15.5	0.21	0.08	0.08	0.05	÷0.01	0.01	0.29	0.1
Malt	8	339.2	77.4	261.8	12.6	13.6	31.2	0.24	0.28	0.29	0.16	0.01	1.76		0.8
	10	318.8	60.5	258.3	14.0	32.6	32.7	0.22	0.18	0.42	0.25	0.02	0.04	1.71	0.8
	13	327.5	70.9	256.6	13.6	24.3	37.0	0.24	0.25	0.39	0.22	0.08	0.05	1.85	0.9
Tystofte Cross barley 6-rd. Steep	0	308.5	283.3	25.2	—	—	13.3	0.22	0.01	0.08	0.03	0.03	0.01	0.19	0.1
Malt	6	288.4	69.2	219.2	6.0	14.1	23.9	0.26	0.19	0.22	0.10	0.00	0.04	1.48	0.7
	8	278.5	65.9	212.6	5.6	24.4	24.9	0.23	0.13	0.31	0.18	÷0.07	0.04	1.46	0.7
	11	280.2	66.5	213.7	5.9	22.4	25.1	0.25	0.18	0.28	0.13	÷0.03	0.03	1.52	0.7
Abed 110 6-rd. Steep	0	220.2	200.0	20.2	—	—	10.4	0.17	0.04	0.10	0.03	÷0.02	0.28		0.1
Malt	6	206.9	50.3	156.6	4.3	9.0	26.1	0.25	0.09	0.21	0.06	0.08	1.37		0.7
	8	196.0	46.3	149.7	6.4	17.8	29.9	0.25	÷0.05	0.31	0.14	0.01	1.64		0.8
	11	186.0	43.5	142.5	7.5	26.7	34.2	0.17	0.02	0.29	0.10	0.08	1.78		0.9
Analytical error c.		2.1		2.6	2.4		3.3	0.07							

[1]) The malt greatly overgrown with micro-organisms at close of experiment: the wort produced was very dark.

ley from Tystofte.

n	$\frac{Q=}{n \div N}$	N	P	T	P+T	p_H	α	Ds	Cv	Pa	Ta	Aml	Rn	Ps	Ex	R'	
								Numerical value of ... process									
								(1)	(2)	(3)	(4)	(5)	(6)				
Total of nitrogen	insoluble compounds	soluble decomposition products	peptic	tryptic	proteolytic		10 000 steeped corns contain — g. nitrogen in form of Albumin II	Proteid solution	Albumin conversion	Peptic action	Tryptic action	Ammonia liberation	Root formation	Peptic action	% extract of dry malt matter	g. rootlets for 100 g. malt grains	hussars
93	6.18	0.75	0.16	0.29	0.45	6.0	0.04	—	—	—	—	—	—	—	6.57	—	
83	3.03	2.80	0.58	1.77	2.35	5.9		3.15	3.00	3.00	2.58	1.12	1.10	0.79	80.19	7.4	some
59	2.75	2.84	0.58	1.85	2.43	5.9		3.43	3.32	3.32	2.90	1.37	1.34	0.79	80.09	10.2	fairly numerous
54	2.76	2.78	0.68	1.78	2.46	5.9		3.42	3.39	3.40	2.90	1.42	1.39	0.71	79.56	11.7	extraord. numerous
13	6.34	0.79	0.10	0.34	0.44	5.9	0.06	—	—	—	—	—	—	—	7.10	—	
95	3.16	2.79	0.54	1.84	2.38	5.9		3.18	3.10	3.12	2.68	1.22	1.18	0.80	79.82	6.8	some
59	2.78	2.81	0.55	1.87	2.42	5.9		3.56	3.48	3.52	3.07	1.59	1.54	0.82	79.63	—	fairly numerous
60	2.72	2.88	0.57	1.95	2.52	5.9		3.62	3.55	3.61	3.14	1.58	1.53	0.86	78.70	11.4	extraord. numerous
93	8.12	0.81	0.19	0.31	0.50	5.7	0.08	—	—	—	—	—	—	—	7.15	—	
91	4.73	3.18	0.64	2.01	2.65	5.8		3.39	3.20	3.17	2.72	1.06	1.02	0.81	77.41	5.8	some few
62	4.33	3.29	0.68	2.09	2.77	5.8		3.79	3.65	3.58	3.09	1.35	1.31	0.86	77.59	7.7	many
55	4.22	3.33	0.64	2.16	2.80	5.8		3.90	3.73	3.68	3.23	1.42	1.38	0.85	76.94	8.8	extraord. numerous
16	6.45	0.71	0.15	0.34	0.49	5.9	0.03	—	—	—	—	—	—	—	7.98	—	
24	3.29	2.95	0.37	1.98	2.35	5.9		3.16	2.82	2.78	2.56	0.99	0.92	0.82	78.20	4.7	many
09	2.86	3.23	0.51	2.20	2.71	5.9		3.59	3.31	3.29	2.93	1.14	1.07	0.96	79.56	6.5	extraord.
00	2.46	3.54	0.63	2.51	3.14	5.9		3.99	3.80	3.81	3.33	1.25	1.16	1.31	79.36	6.5	numerous[1]
18	8.30	0.88	0.15	0.40	0.55	5.7	0.10	—	—	—	—	—	—	—	8.05	—	
33	4.79	3.54	0.65	2.37	3.02	5.9		3.51	3.32	3.32	2.82	0.90	0.85	0.98	78.07	4.9	
29	4.67	3.62	0.72	2.35	3.07	5.9		3.63	3.47	3.41	2.84	0.93	0.89	1.00	78.06	5.7	a good few
13	4.30	3.83	0.88	2.54	3.42	5.8		4.00	3.95	3.92	3.19	1.09	1.05	1.04	77.93	6.1	a good few
35	7.64	0.71	0.12	0.30	0.42	5.6	0.08	—	—	—	—	—	—	—	8.14	—	
84	5.10	2.74	0.46	1.76	2.22	5.9		2.54	2.34	2.31	1.97	—	0.51	0.69	77.20	3.7	few
47	4.63	2.84	0.69	1.75	2.44	5.9		3.01	2.91	2.90	2.33	0.91	0.88	0.73	81.04	4.3	few
62	4.54	3.08	0.69	1.90	2.59	5.9		3.10	2.93	2.90	2.33	0.77	0.73	0.80	78.36	4.2	few
.67	6.10	0.57	0.14	0.20	0.34	5.6	0.01	—	—	—	—	—	—	—	8.16	—	
.26	3.97	2.29	0.32	1.52	1.84	6.0		2.13	1.95	1.91	1.73	0.44	0.41	0.61	76.00	2.1	none
.11	3.83	2.28	0.42	1.50	1.92	5.9		2.27	2.15	2.14	1.86	0.59	0.56	0.59	76.33	2.1	none
.15	3.79	2.36	0.38	1.55	1.93	5.9		2.31	2.14	2.11	1.87	0.54	0.52	0.62	76.28	2.1	none
.64	4.12	0.52	0.11	0.28	0.39	5.8	÷0.04	—	—	—	—	—	—	—	9.15	—	
.48	2.42	2.06	0.35	1.37	1.72	5.9		1.70	1.57	1.49	1.25	—	0.16	0.60	75.68	2.2	some few
.28	1.98	2.30	0.46	1.64	2.10	5.8		2.14	2.15	2.07	1.72	—	0.36	0.71	76.37	3.4	a good few
.20	1.70	2.44	0.47	1.78	2.25	5.8		2.36	2.30	2.30	1.94	—	0.44	0.81	76.59	4.0	fairly numerous
.13	0.12	0.05		0.07		0.1					0.07						

TABLE XXIV. **Year 1914. Barley from Tystofte. For 1000 g. dry matter:**

Columns marked			R_1	Gx_1	S_1	n_1	N_1	AI_1	AII_1	P_1	T_1	$\frac{P_1}{T_1}+$	O_1
Sort		After conclusion of normal steeping, germinated at 14—16° in — days	g. rootlets	g. extract	acid ≙ cc. n. NaOH	g. nitrogen in form of: total of nitrogen	soluble compounds	albumin: I	albumin: II	decomposition products: peptic	decomposition products: tryptic	decomposition products: proteolytic	peptide-bound nitrogen
Tystofte Prentice, 2-rd. with $MgSO_4$ Nr. 1	Steep	0	—	64.0	37.3	17.46	1.89	0.65	0.10	0.40	0.73	1.13	0.45
	Malt	8	62.7	680.1	98.5	14.69	7.05	0.65	0.48	1.46	4.46	5.92	2.44
		10	82.1	652.2	100.3	14.08	7.15	0.65	0.38	1.46	4.66	6.12	2.44
		13	91.2	626.2	99.5	13.96	7.00	0.63	0.18	1.71	4.48	6.19	2.24
Tystofte Prentice, 2-rd. without $MgSO_4$ Nr. 2	Steep	0	—	71.5	43.9	17.89	1.98	0.73	0.15	0.25	0.85	1.10	0.53
	Malt	8	56.7	680.9	97.1	14.93	7.00	0.68	0.35	1.35	4.62	5.97	2.53
		10	—	646.1	101.1	14.03	7.05	0.63	0.35	1.38	4.69	6.07	2.58
		13	91.3	639.8	106.6	14.05	7.23	0.58	0.33	1.43	4.89	6.32	2.68
Tystofte Prentice, 2-rd. Nr. 3	Steep	0	—	71.7	42.5	22.71	2.06	0.58	0.20	0.48	0.79	1.27	0.41
	Malt	8	50.9	674.7	97.4	20.12	8.09	0.66	0.69	1.63	5.11	6.74	2.47
		10	64.8	661.4	102.7	19.38	8.37	0.76	0.56	1.73	5.31	7.04	2.59
		13	70.9	627.9	103.2	19.20	8.47	0.71	0.64	1.63	5.49	7.12	2.57
Svalöf Golden barley 2-rd.	Steep	0	—	78.6	40.2	21.33	2.12	0.57	0.09	0.45	1.01	1.46	0.57
	Malt	8	41.7	671.2	115.6	18.59	8.79	0.69	1.10	1.10	5.90	7.00	3.01
		10	53.9	651.8	120.9	18.14	9.62	0.63	0.92	1.52	6.55	8.07	3.43[1]
		13	51.8	619.6	135.2	17.87	10.55	0.54	0.66	1.88	7.48	9.36	4.47[1]
Binder barley (Abed 411) 2-rd.	Steep	0	—	80.1	41.9	24.03	2.30	0.60	0.26	0.39	1.05	1.44	0.55
	Malt	8	43.2	696.1	103.1	21.81	9.27	0.60	0.76	1.70	6.20	7.90	3.12
		10	50.0	691.9	106.0	21.97	9.48	0.76	0.68	1.88	6.15	8.03	3.17
		13	52.1	659.7	113.1	21.28	10.03	0.68	0.39	2.30	6.65	8.95	3.27
Abed 570 2-rd.	Steep	0	—	81.0	42.3	22.78	1.94	0.57	0.22	0.33	0.82	1.15	0.38
	Malt	8	34.4	714.2	85.1	21.39	7.47	0.65	7.64	1.25	4.80	6.05	2.26
		10	38.2	704.6	89.2	20.38	7.75	0.60	4.91	1.88	4.77	6.65	2.37
		13	37.1	700.0	100.9	20.79	8.40	0.65	6.82	1.88	5.18	7.06	2.56
Tystofte Cross barley 6-rd.	Steep	0	—	84.2	44.4	22.28	1.90	0.74	0.03	0.47	0.67	1.14	0.40
	Malt	6	20.0	732.3	79.8	20.91	7.65	0.87	0.63	1.07	5.08	6.15	2.44
		8	18.7	710.3	83.2	20.41	7.62	0.77	0.43	1.40	5.01	6.41	2.37
		11	19.7	714.0	83.9	20.55	7.88	0.84	0.60	1.27	5.18	6.45	2.47
Abed 110 6-rd.	Steep	0	—	91.0	46.9	20.91	2.34	0.77	÷0.18	0.50	1.26	1.76	0.72
	Malt	6	19.4	705.8	117.6	20.19	9.28	1.13	0.41	1.58	6.17	7.75	3.43
		8	28.8	674.7	134.8	19.29	10.37	1.13	0.23	2.07	7.39	9.46	3.92
		11	33.8	642.2	154.1	18.93	11.00	0.77	0.09	2.12	8.02	10.14	4.37
Analytical error c.			6.4	6.9	8.7	0.34	0.13	0.19					

[1] The malt greatly overgrown with micro-organisms at close of experiment; the wort produced was very dark.

Found for value			a	b	m	c	p	s	q	k	U	h	e	f							g	pH	10 000 barley corns contain g. dry matter	% dry matter in the barley corns	Normal steeping time of the sample in hours
Sort		After conclusion of normal steeping, germinated at 14–16° in ... days[1])	1000 wet corns weigh gr.	% dry matter in the corns	gr. hydrous matter	containing nitrogen ≙ cc. n/10 acid	Quantity of rootlet: Root gr. rootlets	Quantity of rootlet: Root with % dry matter	Quantity of rootlet: Corn g. malt grains	% extract (Ball.	25 cc. dilute wort ≙ no. of corns	25 cc. dilute wort: acid ≙ cc. n/10 NaOH	25 cc. dilute wort: nitrogen ≙ cc. n/10 acid as: total nitrogen	precipitable by: $SnCl_2$	$HgCl_2$	$FeAc_3$	$UrAc_2$	$MgSO_4$	formol-titratable	50 cc. dilute wort contains nitrogen in form of ammonia ≙ cc. n/10 acid				
Binder barley Abed 110 6-rd.	Tystofte Prentice 2-rd.	Steep	0	31.72	93.05	4.1154	48.4	—	—	—	0.87	78.8	1.2	3.4	0.9	1.6	1.6	1.9	1.3	0.7	—	5.7	304.4	89.15	72
		Malt	8	30.52	90.49	4.0304	45.6	7.2893	90.95	111.9	8.02	81.9	3.0	13.2	1.2	3.5	3.7	5.0	2.3	4.3	0.4	5.7			
			10	29.52	91.25	4.0016	45.2	8.9513	92.39	106.4	8.07	84.7	3.2	13.8	1.1	3.5	3.7	5.1	2.3	4.4	0.4	5.7			
			13	28.09	93.81	4.0466	47.6	12.3648	90.08	124.1	8.16	89.0	3.5	14.4	1.1	3.6	3.9	5.5	2.3	4.5	—	5.7			
	Svalöf Gold. barley 2-rd.	Steep	0	32.06	92.14	4.0560	48.4	—	—	—	0.76	78.0	1.2	2.9	0.8	1.4	1.4	1.6	1.1	0.8	0.1	5.9	301.7	89.21	72
		Malt	8	31.35	88.16	4.0595	45.4	7.9770	91.02	116.2	7.76	79.7	2.8	11.3	1.1	3.4	4.1	4.6	2.6	3.6	—	5.9			
			10	29.16	92.12	4.0403	47.7	8.6990	92.82	111.3	8.06	85.7	2.9	12.6	1.0	3.5	4.8	4.7	2.6	3.9	—	5.9			
			13	27.04	89.06	4.0529	47.5	11.1912	90.75	131.6	7.78	92.5	2.9	12.8	1.0	3.6	4.5	5.0	2.5	4.0	0.4	5.9			
	Abed 57° 2-rd.	Steep	0	37.20	93.22	4.0562	44.2	—	—	—	0.75	67.2	1.0	2.9	1.1	1.6	1.7	1.6	1.4	0.6	0.1	5.9	348.0	89.16	48
		Malt	8	34.52	92.51	4.0362	43.3	6.0906	89.33	118.4	8.47	72.4	2.7	13.6	1.1	3.5	4.0	4.6	2.4	4.5	0.5	5.9			
			10	33.16	91.97	4.0000	43.0	7.6612	88.40	126.8	8.37	75.4	2.7	13.3	1.1	3.4	4.4	4.6	2.3	4.3	—	5.9			
			13	31.45	91.84	4.1199	43.7	8.9525	90.84	131.4	8.39	79.5	2.8	13.1	1.0	3.4	4.8	4.7	2.2	4.1	0.4	5.9			
		Steep	0	33.62	93.42	4.0429	51.6	—	—	—	0.84	74.4	1.3	3.2	1.0	1.6	2.0	1.9	1.6	0.8	0.1	5.8	323.6	89.93	48
		Malt	8	31.09	92.94	4.2256	53.4	7.9527	91.95	110.5	8.29	80.4	3.4	15.6	1.3	3.8	4.4	5.8	2.9	5.3	0.5	5.8			
			10	30.68	92.44	4.1140	52.2	9.5351	88.90	116.6	8.22	81.5	3.5	15.9	1.2	3.9	5.1	6.1	3.3	5.1	—	5.8			
			13	28.42	92.13	4.1645	53.0	12.1817	91.14	125.0	8.16	87.9	3.7	16.8	1.3	3.9	5.5	6.1	2.9	5.4	0.5	5.8			
	Abed 110 6-rd.	Steep	0	27.82	91.26	4.0474	62.5	—	—	—	1.05	89.9	1.5	4.2	1.3	2.0	2.2	2.1	1.5	1.2	0.1	5.9	246 6	87.20	72
		Malt	7	26.91	90.89	4.0360	62.7	4.7086	91.11	127.4	7.86	92.9	4.0	21.1	1.7	4.8	5.9	6.6	3.5	7.5	0.9	5.8			
			9	24.59	90.58	4.0181	64.0	6.2381	93.38	108.6	7.81	101.6	4.5	23.1	1.5	4.8	6.5	6.9	3.2	8.3	1.0	5.8			
			11	23.09	90.72	4.0024	65 0	8.8496	90.13	129.7	7.72	108.4	5.1	24.0	1.4	4.6	5.6	7.2	3.0	8.7	0.9	5.7			

[1]) = days of 24 hours.

TABLE XXVI. **Year 19**

Columns marked		A	Y	Gx	R	I	S	A_I	A_{II}	D	E	F	G	H	O
Sort	After conclusion of normal steeping, germinated at 14—16° in . . . days	10 000 corns gave													
		g. dry matter	g. unaffected matter (husk)	g. extract	g. loss as rootlets	g. loss by oxidation	acid ∾ cc. n. NaOH	g. nitrogen in the form albumin I	albumin II	peptic decomposition products = P: Deuteroalbumin	Propepton	Pepton	tryptic decomposition products = T: Ammonia	Amine-Amide	peptide-bound nitrogen
Tystofte Prentice 2-rd.	Steep 0	295.1	272.6	22.5	—	—	15.7	0.17	0.05	0.06	0.01	0.05	0.26		0.1.
	Malt 8	276.1	60.7	215.4	18.1	0.9	36.9	0.20	0.15	0.24	0.04	0.22	0.04	1.36	0.7.
	Malt 10	269.4	59.8	209.6	22.9	2.8	37.4	0.19	0.16	0.23	0.03	0.24	0.04	1.39	0.7.
	Malt 13	263.5	62.3	201.2	29.6	2.0	39.8	0.17	0.15	0.25	0.04	0.26	1.40		0.7
Svalöf Golden barley 2-rd.	Steep 0	295.5	275.6	19.9	—	—	15.0	0.15	0.05	0.05	0.00	0.03	0.01	0.24	0.1
	Malt 8	276.4	62.3	214.1	19.6	÷0.5	34.5	0.19	0.14	0.27	0.12	0.09	1.18		0.6
	Malt 10	268.7	62.1	206.6	21.2	5.6	33.4	0.16	0.06	0.36	0.20	÷0.01	1.29		0.6
	Malt 13	240.8	55.9	184.9	20.9	33.8	31.7	0.15	0.10	0.30	0.13	0.07	0.03	1.16	0.6
Binder barley (Abed 411) 2-rd.	Steep 0	346.8	324.1	22.7	—	—	15.5	0.23	0.05	0.06	0.01	÷0.01	0.01	0.25	0.1
	Malt 8	319.3	61.4	257.9	15.9	11.6	37.0	0.22	0.14	0.32	0.09	0.12	0.05	1.68	0.8
	Malt 10	305.0	60.4	244.6	17.7	24.1	35.7	0.20	0.03	0.40	0.19	0.03	1.61		0.8
	Malt 13	288.8	56.1	232.7	19.5	38.5	34.7	0.18	÷0.04	0.86	0.24	÷0.01	0.04	1.44	0.72
Abed 570 2-rd.	Steep 0	314.1	291.1	23.0	—	—	18.0	0.18	0.04	0 08	0.08	÷0.01	0.01	0.23	0.15
	Malt 8	288.9	62.1	226.8	20.6	4.6	42.0	0.23	0.16	0.26	0.12	0.24	0.04	1.67	0.92
	Malt 10	283.6	61.7	221.9	22.3	8.2	42.4	0.20	0.15	0.32	0.21	0.16	1.70		0.88
	Malt 13	261.9	57.9	204.0	25.2	27.0	41.6	0.20	0.00	0.42	0.26	0.09	0.04	1.67	0.86
Abed 110 6-rd.	Steep 0	253.9	230.0	23.9	—	—	16.9	0.21	÷0.02	0.12	0.04	0.02	0.01	0.31	0.19
	Malt 7	244 6	58.9	185.7	9.1	0.2	43.1	0.26	0.11	0.35	0.16	0.12	0.07	2.11	1.12
	Malt 9	222.8	54.2	168.6	13.2	17.9	44.3	0.20	0.02	0.45	0.22	0.06	0.07	2.16	1.15
	Malt 11	209.2	53.1	156.1	18.4	26.3	47.1	0.19	0.07	0.34	0.13	0.21	0.07	2.09	1.12
Analytical error c.		2.1		2.6	2.4		3.3	0.07							

rley from Abed.

n	$\frac{Q}{n \div N}$	N	P	T	P+T		α	Ds	Cv	Pa	Ta	Aml	Rn	Ps	Ex	R′	
								Numerical value of . . . process									
								(1)	(2)	(3)	(4)	(5)	(6)				
total of nitrogen	insoluble compounds	soluble	decomposition products: peptic	decomposition products: tryptic	decomposition products: proteolytic	p_H.	10 000 steeped corns contain — g. nitrogen in form of Albumin II	Proteid solution	Albumin conversion	Peptic action	Tryptic action	Ammonia liberation	Root formation	Peptic action	% extract of dry malt matter	g. rootlets for 100 g. malt grains	hussars
5.22	4.62	0.60	0.12	0.26	0.38	5.7	0.05	—	—	—	—	—	—	—	7.61	—	
4.83	2.58	2.25	0.50	1.40	1.90	5.7		2.04	1.94	1.91	1.53	—	0.39	0.60	78.02	6.5	many
4.66	2.38	2.28	0.50	1.43	1.93	5.7		2.24	2.13	2.11	1.73	—	0.56	0.60	77.79	8.4	extraord.
4.63	2.36	2.27	0.55	1.40	1.95	5.7		2.26	2.16	2.16	1.73	—	0.59	0.58	76.39	10.0	numerous
5.35	4.82	0.53	0.08	0.25	0.33	5.9	0.05	—	—	—	—	—	—	—	6.72	—	
4.91	2.92	1.99	0.48	1.18	1.66	5.9		1.90	1.81	1.77	1.37	—	0.44	0.48	77.47	6.9	
4.82	2.76	2.06	0.55	1.29	1.84	5.9		2.06	2.05	2.04	1.57	—	0.53	0.49	76.88	7.8	many
4.43	2.49	1.94	0.50	1.19	1.69	5.9		2.33	2.28	2.28	1.86	0.94	0.92	0.45	76.81	8.5	
5.67	5.07	0.60	0.06	0.26	0.32	5.9	0.05	—	—	—	—	—	—	—	6.55	—	
5.18	2.56	2.62	0.53	1.73	2.26	5.9		2.51	2.42	2.43	1.96	0.53	0.49	0.75	80.78	5.1	
4.99	2.53	2.46	0.62	1.61	2.23	5.9		2.54	2.56	2.59	2.03	—	0.68	0.67	80.23	6.0	few
4.67	2.36	2.31	1.09	1.48	2.57	5.9		2.71	2.80	2.85	1.82	1.03	1.00	0.59	80.58	6.8	some
5.01	5.40	0.61	0.15	0.24	0.39	5.8	0.04	—	—	—	—	—	—	—	7.31	—	
4.50	2.78	2.72	0.62	1.71	2.33	5.8		2.62	2.50	2.45	1.98	0.54	0.51	0.77	78.51	7.2	few
4.45	2.71	2.74	0.69	1.70	2.39	5.8		2.69	2.58	2.56	2.02	—	0.56	0.73	78.25	8.2	few
5.07	2.39	2.68	0.77	1.71	2.48	5.8		3.01	3.05	3.03	2.41	0.97	0.94	0.71	77.91	9.7	some
5.02	5.37	0.65	0.14	0.32	0.46	5.9	÷0.02	—	—	—	—	—	—	—	9.40	—	
4.86	2.68	3.18	0.63	2.18	2.81	5.8		2.69	2.56	2.51	2.02	0.22	0.16	0.93	75.93	3.7	
4.49	2.31	3.18	0.73	2.23	2.96	5.8		3.06	3.02	3.03	2.44	0.59	0.53	0.96	75.69	5.7	some few
4.24	2.14	3.10	0.68	2.16	2.84	5.7		3.23	3.14	3.16	2.62	0.84	0.78	0.93	74.62	6.8	
0.13	0.12	0.05		0.07		0.1					0.07						

TABLE XXVII. **Year 1914. Barley from Abed. For 1000 g. dry matter:**

Columns marked			R_1	Gx_1	S_1	n_1	N_1	AI_1	AII_1	P_1	T_1	P_1+T_1	O_1
Sort		After conclusion of normal steeping, germinat. at 14-16° in — days	g. rootlets	g. extract	acid ∾ cc. n. NaOH	g. nitrogen in form of: total of nitrogen	soluble compounds	Albumin I	Albumin II	decomposition products: peptic	decomposition products: tryptic	decomposition products: proteolytic	peptide-bound nitrogen
Tystofte Prentice, 2-rd.	Steep	0	—	73.9	51.6	17.15	1.97	0.56	0.16	0.39	0.85	1.24	0.43
	Malt	8	59.5	707.6	121.2	15.87	7.39	0.66	0.49	1.64	4.60	6.24	2.40
		10	75.2	688.5	122.9	15.31	7.49	0.62	0.53	1.64	4.70	6.34	2.40
		13	97.2	660.9	130.7	15.21	7.46	0.56	0.49	1.81	4.60	6.41	2.33
Svalöf Golden barley 2-rd.	Steep	0	—	66.0	49.7	17.74	1.76	0.50	0.17	0.27	0.83	1.10	0.50
	Malt	8	65.0	709.7	114.4	16.28	6.60	0.63	0.46	1.59	3.91	5.50	2.09
		10	70.3	684.9	110.7	15.98	6.83	0.53	0.20	1.82	4.28	6.10	2.12
		13	69.3	612.9	105.1	14.69	6.43	0.50	0.33	1.66	3.94	5.60	1.99
Binder barley (Abed 411), 2-rd.	Steep	0	—	65.2	44.5	16.30	1.72	0.66	0.14	0.17	0.75	0.92	0.37
	Malt	8	45.7	741.2	106.3	14.89	7.53	0.63	0.40	1.52	4.97	6.49	2.53
		10	50.9	703.0	102.6	14.34	7.07	0.57	0.09	1.78	4.63	6.41	2.30
		13	56.0	668.8	99.7	13.42	6.64	0.52	÷ 0.11	3.13	4.25	7.38	2.07
Abed 570, 2-rd.	Steep	0	—	71.1	55.6	18.57	1.88	0.56	0.12	0.46	0.74	1.20	0.46
	Malt	8	63.7	700.8	129.8	17.00	8.40	0.71	0.49	1.92	5.28	7.20	2.84
		10	68.9	685.7	131.0	16.84	8.47	0.62	0.46	2.13	5.25	7.38	2.72
		13	77.9	630.4	128.5	15.67	8.28	0.62	0.00	2.94	5.28	8.22	2.66
Abed 110, 6-rd.	Steep	0	—	96.9	68.5	24.41	2.64	0.85	÷ 0.08	0.57	1.30	1.87	0.77
	Malt	7	36.9	753.0	174.8	23.76	12.89	1.05	0.45	2.55	8.84	11.39	4.54
		9	53.5	683.7	179.6	22.26	12.89	0.81	0.08	2.96	9.04	12.00	4.66
		11	74.6	633.0	191.0	21.25	12.57	0.77	0.28	2.76	8.76	11.52	4.54
Analytical error c.			6.4	6.9	8.7	0.34	0.13	0.19					

H. Schjerning.

NIELS CHRISTIAN HENRIK SCHJERNING, born 22 July 1862 in the island of Falster, Denmark, died 9 November 1914 in Copenhagen, commenced as a pharmacist, subsequently studying chemistry at the Polytechnic Institute of Copenhagen; was from 1 Oct. 1887 to 31 Oct. 1890 Assistant at the chemical laboratory of the Royal Veterinary and Agricultural College, and thereafter appointed Chemist to the New Carlsberg Brewery. Here he planned and commenced his great work: the Proteïd Substances of Barley, in the Grain itself and during the Brewing Processes, which he concluded — having, meanwhile, on the 1st May 1904, been nominated Superintendent Extraordinary under the Chemical Department of the Carlsberg Laboratory — after more than 20 years of research. His loss will be deeply felt by the Carlsberg Laboratory

LIST OF WORKS PUBLISHED BY H. SCHJERNING.

1888. A Rapid Method for Quantitative Determination of Calcium and Magnesia, especially in Guncotton. Tidskr. f. Physik og Chemi II, **9**, 353—356.

1890. A contribution to the Chemistry of Manganese. Overs over Vidsk. Selskabs Forhandl. 1890. 311—329.

1892. Einige Phenylhydrazinverbindungen mit Metallsalzen der Magniumgruppe. Journal f. prakt. Chemie. N. F. **47**, 80—88.

1893. Ueber die quantitative Trennung der in Bierwürze enthaltenen, amorphen, stickstoffhaltigen organischen Verbindungen. Zeitschr. f. analyt. Chemie **33**, 263—299.

1894. Untersuchungen über die in der Bierwürze vorhandenen stickstoffhaltigen organischen Verbindungen. ibid **34**, 135—147.

1896. Noch einige Bemerkungen über die quantitative Trennung der in Bierwürzen vorhandenen Proteïnstoffe. ibid. **35**, 285—296.

1897. Beiträge zur Chemie der Proteïnfällungen. ibid. **36**, 643—663.

1897. Weitere Beiträge Zur Chemie der Proteïnfällungen. ibid. **37**, 73—87.

1898. Methode zur quantitativen Bestimmung der verschiedenen Proteïnindividuen in Bierwürze und anderen Proteïnlösungen. Zeitschr. f. analyt. Chemie **37**, 413—422.

1900. Einige kritische Untersuchungen über die quantitativen Fällungsverhältnisse verschiedener Proteïnfällungsmittel. ibid. **39**, 545—566.

— Ueber die Bestimmung des Proteïngehaltes in Futtermitteln. ibid. **39**, 633—639.

1905. On the Proteïd substances of Barley, in the grain itself and during the brewing processes.

I. On the formation and transformation of Proteïd substances during the growth, ripening and storage of barley. Compt.-rend. des travaux du Laboratoire de Carlsberg. **6**, 229—307.

1909. II. Transformation of the proteïd matters during malting and malt storing. ibid. **8**, 169—401.

1912. III. Transformation of the proteïd matters during mashing, boiling of wort, and fermentation. ibid. **9**, 237—396.

1914. Summary view. ibid. **11**, 45—105.

1916. H. Schjerning and Jenny Hempel:

IV. Investigations as to malting power of various sorts of barley. ibid. **11**, 333—378.

COMPTE RENDU SUR LA DONATION HANSEN

PAR L'ADMINISTRATION DE LA DITE DONATION.

A la date du 7 septembre 1904, le professeur EMILE-CHRISTIAN HANSEN, Dr, chef du Service de microbiologie du Laboratoire de Carlsberg, et son épouse MATHILDE, née MELCHIOR, dressèrent un acte de dernière volonté dans lequel, ainsi que dans une minute antérieure (datée du mois d'août 1904) qui s'y rattache, les motifs qui les portaient à instituer la Donation se trouvent exposés en ces termes:

«Comme c'est au succès dont j'ai été favorisé dans mes recherches microbiologiques que je dois ma fortune, je désire qu'une partie de cette fortune soit employée à aider ceux qui, après moi, voudront pousser plus avant les recherches dans ce domaine. Dans ceux-là je comprends non seulement mes compatriotes, mais, au même point, tous les étrangers.»

Après la mort du professeur E.-Chr. Hansen, survenue en 1909 et suivie de près de celle de Madame Hansen, l'administration de leur succession fut confiée à M.M. Carl Jacobsen, Dr, brasseur, et S. P. L. Sörensen, professeur et Dr, comme exécuteurs testamentaires, auxquels fut adjoint en qualité de syndic M. J. L. Nathansen, avocat à la cour d'appel de Copenhague.

Sur les bases des dispositions du testament, et de concert avec le fils des testateurs M. Chr.-Mathæus Hansen, qui témoignait d'un vif intérêt à la création de la Donation, on en élabora la fondation qui, à la date du 17 juin 1911, obtint la confirmation du Roi sous la désignation, indiquée dans le testament, de «Prix d'encouragement aux travaux de microbiologie, institué par le professeur Emile-Chr. Hansen». La fondation se trouve notifiée dans le Bulletin des lois et des mesures administratives A de 1911, page 196.

En ce qui concerne l'emploi de la Donation Hansen, les Statuts portent ceci:

Les intérêts des fonds qui constituent ce legs, déduction faite des dépenses nécessaires, seront employés à des intervalles convenables — soit, à l'ordinaire, tous les deux ou trois ans —, à l'attribution d'un prix d'encouragement à l'auteur d'un travail important d'ordre microbiologique publié, en Danemark ou à l'étranger, depuis la dernière attribution du prix (pour la première attribution, depuis la mort du donateur).

Le prix d'encouragement, décerné le 8 mai, jour anniversaire du donateur, consiste en une médaille d'or portant l'effigie du donateur et à laquelle sera jointe la somme de 2 000 couronnes danoises. Après le décès de certaines personnes nommées dans les Statuts (1), l'Administration du legs aura le droit d'augmenter le montant du prix. Par exception, l'Administration pourra aussi prélever sur les fonds du legs, une petite subvention à de jeunes savants s'occupant spécialement de recherches microbiologiques. Le prix sera décerné pour la première fois en 1915, au plus tard.

Six mois avant la date de l'attribution du prix, il sera inséré dans un on plusieurs ouvrages périodiques du Danemark, de France, d'Angleterre, d'Allemagne ou d'autres pays, au choix de l'Administration de la Donation, une notification relative au décernement du prix, contenant une invitation à lui transmettre dans un délai déterminé, et en deux exemplaires au moins, les travaux concourant pour ce prix, lesquels devront être écrits dans une des langues scandinaves, ou bien en français, anglais ou allemand. Toutefois, le prix pourra aussi être décerné à des personnes qui ne l'auront pas sollicité.

L'Administration de la Donation se compose normalement, d'après les Statuts, des deux chefs de Service du Laboratoire de Carlsberg, auxquels sera adjoint un biologiste danois élu par la Direction de cet Institut.

Les travaux seront jugés par la Commission des prix, dans laquelle siégeront les Membres de l'Administration de la Donation, ainsi que deux ou plusieurs savants étrangers, spécialistes dans le domaine de la microbiologie.

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(1) En vertu du testament, les sœurs de M. Hansen recevront à perpétuité une somme annuelle fixée d'avance.

Le capital de la Donation, à l'époque de sa fondation, se montait à 44 000 couronnes environ. Peu de temps après la mort de Hansen, le capital fut augmenté d'une somme d'un peu plus de 18 000 couronnes, que lui assigna un comité qui s'était constitué avec l'intention d'ériger en son honneur un monument devant le Laboratoire de Carlsberg, qui avait vu s'accomplir l'œuvre de sa vie. Dans son invitation à souscrire des cotisations, le Comité avait indiqué que les sommes qui seraient souscrites en réponse à cet appel, au delà du montant requis pour l'érection du monument, iraient grossir le fonds de la Donation Hansen (2).

Les fonds supplémentaires que nous venons de mentionner furent incorporés à la fortune de la Donation, par une fondation complémentaire confirmée par le Roi à la date du 23 octobre 1913, laquelle a été notifiée dans le Bulletin ministériel A 1913 et imprimée dans l'Annexe.

Dans la première Administration de la Donation entrèrent M.M. le professeur C. O. Jensen, Dr en méd., élu par la Direction du Laboratoire de Carlsberg, le Dr Johannes Schmidt, chef du Service de microbiologie du Laboratoire de Carlsberg, et le professeur S. P. L. Sörensen, Dr, chef du Service de chimie de cet Institut. L'Administration s'est constituée sous la présidence du professeur Sörensen.

Dans une séance tenue le 30 janvier 1913, l'Administration décida que la médaille susmentionnée, modelée par le sculpteur L. Brandstrup, serait décernée pour la première fois à un savant qui se serait distingué par des recherches sur les êtres microbiens pathogènes (morphologie, biologie et mode d'action des micro-organismes susceptibles de provoquer des maladies chez l'homme et les animaux). En même temps on annonçait l'intention d'inviter à se joindre à la première Commission des prix M. le professeur Calmette à Lille, M. Gaffky, Geh. Obermedicinalrath, à Berlin, et M. le professeur Theobald Smith à Boston. Ces savants se déclarèrent prêts à y entrer.

La Commission des prix, constituée sous la présidence du professeur C. O. Jensen, résolut à l'unanimité de décerner le prix à M. Jules Bordet, professeur de bactériologie à l'Université de Bruxelles et directeur de l'Institut Pasteur du Brabant a Bruxelles.

(2) Voir les Comptes-rendus du Laboratoire de Carlsberg, **10** [123] (1913).

M. le professeur Bordet ayant bien voulu se rendre à Copenhague, une solennité fut célébrée le 8 mai 1914 à l'École Polytechnique, où la médaille ainsi que le prix (de 2 000 couronnes) lui furent remis.

En décembre 1915.

L'Administration de la Donation Hansen :

C. O. Jensen. **Johs. Schmidt.** **S. P. L. Sørensen.**

J. L. Nathansen,
Secrétaire de la Donation.

COMPTES-RENDUS

DES TRAVAUX

DU

LABORATOIRE DE CARLSBERG

12ME VOLUME.

ÉDITION FRANÇAISE.

1915—1917

COPENHAGUE

EN COMMISSION CHEZ H. HAGERUP

IMPRIMERIE DE THIELE

1917

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STUDIES ON PROTEINS.

BY

S. P. L. SÖRENSEN.

THE great development which has taken place in colloidal chemistry during the past decade has had an extraordinary influence upon the study of proteins, and in a few cases this new branch of science has shown, or at least indicated, the way to the explanation of problems as to which we have been hitherto in the dark. In other domains of the study of proteins, however, colloidal chemistry has, in my opinion, not contributed to further progress, but rather the reverse. This will apply more particularly to the comprehension of such chemical or physico-chemical questions with regard to which many solutions of proteins are more closely related to the real solutions than to the colloidal suspensions, and this will generally be the case as soon as we have to deal with properties or reactions in which the nature of the substance in the disperse phase is of some consequence. The fact is that modern colloidal chemistry pays very little regard to the chemical nature of the disperse phase, and the reason for this may probably be looked for in the developmental history of colloidal chemistry.

In his classical works, which laid the foundation of colloidal chemistry, Thomas Graham[1]) strongly emphasizes the difference between colloidal and crystalloidal matters. "I may be allowed", says Graham in the concluding summary of the paper cited (p. 220), "to advert again to the radical distinction assumed in this paper to exist between colloids and crystalloids in their intimate molecular constitution. Every physical and chemical property is characteristically modified in each class. They appear like different worlds of matter, and give occasion to a corresponding division of chemical science. The distinction between these kinds of matter is that subsisting between the material of a

[1] Philos. Transact. Roy. Soc. London, **151**, 183 (1861).

mineral and the material of an organized mass". Graham had, however, a clear understanding that this sharp distinction between colloids and crystalloids was hardly so absolute as he expressed it in the words quoted. In fact, in the same chapter he states as exceptions from the rule, that ice has a colloidal character at and in the neighbourhood of its melting point, while the so-called blood-crystals (hæmoglobin), which consist of a protein, assume crystalline shape. "Can any facts", exclaims Graham in the last lines of the chapter (p. 223), "more strikingly illustrate the maxim that in nature there are no abrupt transitions, and that distinctions of class are never absolute?"

The boundary line between colloids and crystalloids was further effaced in the course of time, as chemists gradually succeeded in preparing an increasing number of substances in the colloidal state and in bringing such marked colloids as the real albumins into crystalline form. It is, however, only the improved, or entirely new methods devised of late years for the preparation of colloidal solutions which have made it possible to prepare such a variety of colloidal solutions of substances of, so to say, any nature that P. P. von Weimarn could with perfect justice describe "der kolloide Zustand als allgemeine Eigenschaft der Materie"[1]).

Looking at it from such a historical point of view, it will be easily understood that colloidal chemistry has gradually been led to occupy itself mainly with the colloidal state of the matter; rarely with the colloidal substance itself. Colloidal chemistry is, as Wo. Ostwald expresses it, "nicht die Lehre von den kolloiden Stoffen, sondern vielmehr die Lehre vom kolloiden Zustand der Stoffe"[2]).

As, moreover, a large number of the most important colloido-chemical researches were made with typical suspensoids, for which class of colloids the nature of the substance is, in general, of slight importance, it is not to be wondered at that the composition and chemical character of the colloidal matter was at last thrown almost completely into the shade. "So mannigfaltig und merkwürdig der Einfluss der fällenden Ionen ist", says H. Freundlich[3])

[1]) Journ. d. russ. chem. Gas. (Febr. 1906); cited after P. P. von Weimarn: Grundzüge der Dispersoidchemie (Dresden 1911), p. 6.

[2]) Wo. Ostwald: Grundriss der Kolloidchemie (Dresden 1909), p. 44.

[3]) H. Freundlich: Kapillarchemie und Physiologie (Dresden und Leipzig 1914), p. 20.

when mentioning the precipitating effect exerted by electrolytes on colloids, especially suspensoids, "so gering ist der der suspendierten Substanz. Man erhält praktisch dieselbe Folge der Fällungswerte, mag man mit einer Suspension von groben Quarzteilchen, mit einer Mastixemulsion, mit kolloiden Lösungen von Platin, Arsentrisulfid oder Berlinerblau arbeiten."

We must, however, be very cautious in drawing conclusions about colloids in general from the results obtained by studies of pronounced suspensoids; conclusions which are to hold good of the typical emulsoids also. Such summary treatment of substances of quite different nature is legitimate only when dealing with properties or reactions contingent upon the physical state of the substance, but not upon its chemical nature. It is, indeed, in the explanation of several phenomena of this kind that the researches on proteins have so richly profited by the results of the colloidal chemistry. But when the latter sometimes tries through the medium of "capillary" chemistry — using a designation proposed by Freundlich, which appropriately limits the domain of the modern researches on colloidal chemistry — to explain the essential, or even all the properties of a protein solution, I believe that colloidal chemistry will in this case do more harm than good. It is, in fact, significant that, to account for the results arrived at, the leading works published of late years concerning the proteins — I need here only mention the pioneer works of Wo. Pauli and his collaborators — have recourse to points of view not restricted to colloidal chemistry, but also to those of common and physical chemistry.

This is not astonishing if we consider in how many points the suspensoid colloids resemble the rough suspensions, while the majority of protein solutions and other typical emulsoid solutions in many ways approach the real solutions. The degree of dispersity, which to the mind of the ingenious organiser of modern colloidal chemistry, Wo. Ostwald, is of paramount importance in well-nigh all colloido-chemical processes, cannot in my opinion in all points give a satisfactory explanation of the great difference which exists between suspensoid and emulsoid colloids. A gold suspension, for instance, has equally pronounced suspensoid properties, whether the disperse phase contains particles of ultra-microscopic or amicroscopic size, while an albumin solution always has the character of an emulsoid, and will only lose this

character when the albumin — for instance by heat-denaturation — is transformed into quite another substance, which in many ways has the character of a suspensoid. Neither do I think that the often rather hypothetical assumption, that the disperse phase is solid in suspensoid but fluid in emulsoid systems, is able to explain the difference mentioned. Such aqueous colloidal solutions of oil, for instance, as were examined by K. Beck[1]) and G. S. Walpole[2]) have a completely suspensoid character, and it is hardly possible in this case to suppose a solid disperse phase.

I think it is far more reasonable to refer the difference between the suspensoids and the emulsoids to a different degree of reaction power between the internal and the external phase[3]). In the case of the typical suspensoids, the reaction between the disperse phase and the dispersion medium is almost exclusively of a capillary-chemical nature — whether the disperse phase be solid or fluid —, and accordingly the most important properties of the system are determined (for instance the difference in electrical charge between the internal and the external phase; conditions of adsorption, and so on). A typical emulsoid, such as albumin, likewise reacts capillary-chemically upon the external phase; but this effect is of more or less subordinate importance. the emulsoid reacting also completely chemically on the external phase in perfect accordance with our present view of the conditions of things as existing in real solutions. It is quite possible that the degree of dispersity of the emulsoid is of great consequence with regard to the question as to which of the two functions here mentioned will be predominant and therefore determinative of the character of the system. An emulsoid system with a high degree of dispersity will probably be almost exclusively characterized by the reaction of the internal phase on the external phase, and consequently approach the real solutions, while the capillary-chemical conditions will probably be of great consequence in emulsoid systems with a low degree of dispersity.

[1]) Zeitschr. physlk. Chem., **58**, 409 (1907).

[2]) Journ. of Physiol., **47** (18. Okt. 1913); Biochemical Journal, **8**, 170 (1914).

[3]) Similar views are met with more or less distinctly pronounced in several places in the literature; see for instance G. Ciamician: Zeitschr. physik. Chem., **69**, 100 (1909); Fil. Bottazzi: Kolloidchem. Beihefte, **3**, 164 (1912); R. Höber: Physikalische Chemie der Zelle und der Gewebe, vierte Auflage, Leipzig und Berlin, 1914, p. 276 and 305.

Thus it appears that this view permits, and even requires, a quite smooth transition from the real solutions through the emulsoids to the suspensoids. To avoid mistakes, it must be expressly emphasized that the names suspensoids and emulsoids, here and in the following, are used in the signification just explained, which on the whole coincides with the prevailing, typical meaning of these terms, save that the name does not predicate anything as to whether the internal phase is solid or fluid.

The main differences between the typical suspensoid and the typical emulsoid systems will therefore in my opinion (which in the main agrees with the views set forth by A. A. Noyes[1]) be the following:

Suspensoid systems show a viscosity differing but little from that of the pure external phase The system generally shows a well-marked difference in electrical charge between the internal and external phases. Only comparatively small concentrations of electrolytes are required to bring about coagulation, which is in most cases irreversible.

Emulsoid systems show a great viscosity and power of foam-formation. The system commonly does not show any marked difference in electrical charge between the internal and external phases. Great concentrations of electrolytes are commonly necessary to bring about coagulation, which is in most cases reversible.

Transition systems exhibit of course corresponding alterations in their properties.

It is not my intention with these few and brief remarks to give a thorough description of modern colloidal chemistry generally, and still less to set forth any new principle of classification for colloidal solutions; my intention is simply to explain the views on the basis of which I have tried to account for the results obtained by extensive researches during the last five years with a typical emulsoid colloid, *hens' egg-albumin*. Colloidal chemistry in the shape which has been given it by its energetic champion Wo. Ostwald offers, no doubt, to protein study a system organized with great talent, but exact experimental investigation has not been able to keep up with the systematic treatment, and therefore the value of the contents does not always correspond with the perfection of the system itself. Thus I disagree with

[1] Journ. Americ. Chem. Soc., **27**, 85 (1905).

Ostwald, who (as is the case several times in the second edition, not yet completed, of "Grundriss der Kolloidchemie") warns us against a comparison of the circumstances in colloidal and real solutions. In the case of albumin solutions, and doubtless also several other typical emulsoid systems, such a comparison between the properties of the colloidal solution and those of a real solution is of the greatest significance for the right understanding of the character of the colloidal solution. Indeed, I think it is even possible to go one step further and to say that the study of real solutions may derive considerable advantage from the results which an exact research of well-defined protein solutions can give, the colloidal character of these permitting the use of research-methods — I refer especially to the use of semi-permeable membranes —, which, when dealing with real solutions, can only be used in exceptional cases and under especial circumstances.

In the researches on egg-albumin solutions here mentioned, which are described in detail in the following series of papers, I have therefore endeavoured to consider the case from a colloido-chemical as well as from a physico-chemical and a purely chemical standpoint. My view of the properties of protein solutions coincides in many ways with that of Wo. Pauli, whose comprehensive and very important researches on the proteins published in the course of the last years are partly parallel to those made here in our laboratory. Pauli and his co-workers, however, have extended their investigations to a great number of substances, whereas I have found it best to confine investigations for the present to a single substance, hens' egg-albumin. In return, it has proved possible to deepen this research, which has not yet been brought to a close, in such a way that the results obtained may be said to rest on a broad and safe experimental basis.

In planning these researches on the lines indicated above, I was led by the consideration that a research (based as far as possible upon exact quantitative chemical and physico-chemical measurements) of a single, but well-defined protein would be of importance not only for protein chemistry, but also for the explanation of the properties of typical emulsoids generally.

In works on chemistry, the concentration of a dissolved substance is usually described as the weight or the number of gram-equivalents of the substance in a certain volume of the solution. This mode of expression is chosen from motives of convenience, and is eminently suitable in the case of concentrations so low as to render the volume of the substance minimal as compared with that of the solvent. Where this is not the case, however, it will often be found convenient to define the concentration of the substance in another way, for instance as the weight of the substance expressed in the unit of weight either of the solution or of the solvent, or as the number of gram-equivalents of the substance in a certain volume of the solvent. It is very easy to find among the works of later years, instances of the advantages derived from using a mode of calculation such as that here mentioned. I need only refer to the well-known researches of Morse, Frazer and Dunbar[1]) on the dependence between the concentration and the osmotic pressure of solutions of cane sugar, and to Kendall's[2]) discussion of the question as to application of the Law of Dilution to strong solutions of weak electrolytes. These works show strikingly, how necessary it is to take into consideration the volume of the substance dissolved, if the same laws are to be used for strong as for weak solutions.

The fact here mentioned will acquire even greater significance when dealing with substances which in a resolved — dissociated or undissociated — state are combined with part of the solvent, in which case the proportion between the quantities of substance dissolved and of solvent is displaced.

After the investigations made in recent years there can hardly be any doubt but that many substances, both undissociated (*e. g.* cane sugar) and dissociated (*e. g.* the ions of the common acids, bases and salts), in aqueous solution are combined with greater or smaller quantities of water. A few years ago, Edward W. Washburn[3]) gave an excellent general view of the experiments carried out for elucidating this question, and later researches have further borne out the supposition that in aqueous solution a sometimes far-reaching hydratation will often take place.

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[1] H. N. Morse and J. C. W. Frazer: Americ. Chem. Journ., **34**. 1 (1905), and H. N. Morse, J. C. W. Frazer and P. B. Dunbar: *Ibid.*, **38**. 175 (1907).

[2] J. Kendall: Journ. Americ. Chem. Soc., **36**, 1069 (1914).

[3] Jahrbuch der Radioaktivität und Elektronik, **5**, 493 (1908), and **6**, 69 (1908).

In such emulsoid solutions as those referred to in the following series of papers, the dispersed substance (in our case the egg-albumin) exists in a hydratized state, the internal phase consisting of egg-albumin in more or less loose connection with greater or smaller quantities of the component parts of the external phase. We have therefore been forced to take into consideration the volume of both of the two phases, and as it appears from our experiments that the composition of the internal phase (hereafter often designated briefly as *egg-hydrate* or *protein-hydrate*) depends on the amount of salt and the hydrogen-ion concentration of the external phase, we have found it convenient to adopt the following plan in our indications relating to concentration.

Suppose, for example, the analysis of an egg-albumin solution containing ammonium sulphate has given the result that 100 g. of the solution contain p g. protein nitrogen and a g. ammonia nitrogen, and suppose further that x is the factor by which the protein nitrogen has to be multiplied to give the quantity by weight of hydrous but ammonium-sulphate-free egg-hydrate, while y is the factor by which the protein nitrogen has to be multiplied to give the weight of ammonium sulphate combined with the egg-hydrate. As the ammonia nitrogen multiplied by 4.7163 gives the weight of the equivalent quantity of ammonium sulphate, it then follows that

100 g. solution will contain

$p \cdot (x + y)$	g. egg-hydrate (internal phase)	
$(4.7163 \cdot a \div p \cdot y)$	g. ammonium sulphate	(external
and $(100 \div p \cdot x \div 4.7163 \cdot a)$	g. water	phase)

The ammonium sulphate concentration, S, of the external phase will accordingly be generally expressed as the weight of ammonium sulphate per 100 g. water in the external phase, and hence we have

$$S = \frac{100 \cdot (4.7163 \cdot a \div p \cdot y)}{100 \div p \cdot x \div 4.7163 \cdot a}.$$

By the egg-hydrate concentration, E, we understand the number of milligram-equivalents of protein nitrogen per 100 g. water in the external phase,

$$\text{so } E = \frac{100 \cdot p}{0.01401 \cdot (100 \div p \cdot x \div 4.7163 \cdot a)}.$$

On occasions where it is desirable to state the egg-hydrate concentration in proportion to the volume of the external phase, E will consequently signify the number of milligram-equivalents of protein nitrogen in that volume of external phase. V_S, which contains 100 g. water and S g. ammonium sulphate. If d_S is the specific gravity of such a solution of ammonium sulphate, we shall have

$$V_S = \frac{100 + S}{d_S},$$

and the egg-hydrate concentration calculated for unity of volume of the external phase — it is convenient to express it in this way for instance in measurements of osmotic pressure —, will therefore be

$$\frac{E}{V_S}.$$

The above expression contains two unknown and not easily determinable quantities, namely x and y. It has been one of the main problems of our investigations to determine the variation of these two factors as dependent on the composition of the external phase, and even if we have not in all points succeeded in surmounting the difficulties which such an investigation presents, still I venture to believe that the approximate values of x and y which we have been able to calculate from our experimental results will render possible a real quantitative investigation into the equilibrium between the two phases of the solution (see further Paper I, p. 39, and Paper V).

Closely connected with this problem is a whole series of questions concerning the crystallisation of egg-albumin by adding sufficient quantities of ammonium sulphate. There may here be some question as to the composition of the precipitate which has crystallised out; its contents of water, of ammonium sulphate or of "surplus" sulphuric acid or ammonia. There may further be question as to the character of the crystallisation process itself and the nature and extension of the alterations which take place before and during crystallisation in the composition and degree of dispersity of the internal phase.

Question may also arise regarding the equilibrium between the precipitate crystallised out and the surrounding mother liquor; the dependence of this equilibrium on the conditions of temperature, salt and hydrogen-ion concentration; the velocity with which

the equilibrium is established, and finally, as to whether the equilibrium found agrees with Gibbs' Phase Rule. Through the investigations described in the following we have likewise tried to give a contribution towards answering the questions here suggested, and we venture to hope that the results obtained will in not inessential points be of importance for the insight into the questions mentioned.

It would carry us too far, if we were in this brief introduction to give anything like a complete account of the method by which these investigations were carried out, and the results which we have arrived at; as regards such details I must refer to the particular papers and confine myself to giving here a summary view of the contents.

Paper I gives a description of the method used in the preparation of the experimental material, and of the tests for purity and the control-researches which we have made in order to prove the fitness for use of the method employed. Further, the analytical methods used are described together with the corresponding control determinations.

Paper II treats of the question as to the capacity of the egg-albumin to combine with acids or bases, especially the capacity to combine with sulphuric acid in the presence of different concentrations of ammonium sulphate.

Paper III discusses the crystallisation of the egg-albumin when ammonium sulphate is added, and gives a contribution towards the elucidation both of the crystallisation process itself and of the question as to composition of the crystals separated.

Paper IV relates to the equilibrium between the egg-albumin crystallised out and the surrounding mother liquor.

Paper V deals with the question as to the osmotic pressure of egg-albumin solutions and its dependence on the composition of the solution. Closely connected with this is the question of variation in the above mentioned factors x and y (see p. 8) with the composition of the external phase.

Later on, we hope in some ensuing papers to be able to give the results of experiments not yet finished on specific gravity, viscosity and heat-denaturation of such ammonium-sulphate-containing egg-albumin solutions as those above mentioned. We have moreover long since made a series of preliminary experiments with other salts than ammonium sulphate, especially ex-

periments on crystallisation with other sulphates and osmotic measurements with egg-albumin solutions containing ammonium chloride, whereas we have not yet made experiments with other proteins than hens' egg-albumin.

In choosing the above mentioned classification of our experimental material, I have been led solely by considerations of expediency, having found that this classification gives a good view of the material experimented upon; but the classification implies nothing as to the consecutive order in which the researches were executed.

We shall frequently have to refer to the preceding or following papers, one research-series entering into and utilising the results arrived at in another. It is even possible that the continued investigations now in progress will necessitate a re-calculation of some of the numerical results now published. However, if in spite of this I have resolved upon publication at the present time, the reason is that the material in hand has reached such an extent that it has become necessary to sift and sum up the results obtained, not least with a view to determining upon which points continued, supplementary investigations would be desirable.

The investigations here mentioned were in their main features planned and executed by Miss Margrethe Høyrup and myself. In a series of especial researches, we have, however, benefited by the excellent assistance of Mr. S. Palitzsch and Miss Jenny Hempel, who have carried out nearly all the electrometric measurements of hydrogen-ions; further of Mr. J. A. Christiansen, whom we have in a great measure to thank for the final elaboration of the osmotic measuring method, and finally of Mr. S. Goldschmidt, who undertook the greater part of the measurements of osmotic pressure.

I have great pleasure in offering my co-workers once more my sincere thanks for the care and ability with which each has contributed towards these somewhat trying investigations.

November 1915.

STUDIES ON PROTEINS.

I. ON THE PREPARATION OF EGG-ALBUMIN SOLUTIONS OF WELL-DEFINED COMPOSITION, AND ON THE ANALYTICAL METHODS USED.

BY

S. P. L. SÖRENSEN AND MARGRETHE HÖYRUP.

A. Purification of the Egg-albumin from Ash, Mucoid and Conalbumin by Crystallisation.

THE purpose here in view was to remove, by crystallisation, the impurities of the egg-albumin solution freed from globulin. We have especially fixed our attention upon the ash, the amount of nitrogenous non-coagulable substances which we comprise under the name "*Mucoid*" and the amount of nitrogenous coagulable but non-crystallisable substances which we comprise under the name "*Conalbumin*".

a. Method of Crystallisation.

To bring about the crystallisation of the egg-albumin we have adopted, in its essential features, the well-known method of F. G. Hopkins and S. N. Pinkus[1]), having effected the precipitation by means of ammonium sulphate and diluted sulphuric acid. In order to obtain an effective purification, we have, after filtering off the precipitate crystallised out, washed this with an ammonium sulphate solution of proper strength.

The method here employed, then, is as follows: The whites of 60 fresh-laid eggs (about 2 l.) are stirred briskly together with an equal bulk of a saturated solution of ammonium sulphate, and the precipitate filtered off. To the clear, reddish-yellow filtrate (about 3300 cc.) is added saturated ammonium sulphate solution till incipient permanent turbidity (about 140 cc.) is reached, and

[1]) Journ. of Physiol. **23**, 130 (1898).

it is then determined how much $^n/_5$ sulphuric acid is necessary to give a measured sample of the filtrate a hydrogen-ion concentration favourable to crystallisation. By addition of $^n/_5$ sulphuric acid the above mentioned turbidity is first dissolved, then the colour of the solution changes from reddish-yellow to yellow, and at last an amorphous voluminous precipitate is separated, which can at first be dissolved very easily by stirring or shaking. but later becomes more and more difficult to dissolve. The quantity of acid to be added to the whole filtrate must now, in order to give the solution a hydrogen-ion concentration favourable to crystallisation, be chosen in such a way that the amorphous precipitate separated by the addition of acid can be just dissolved again by stirring or shaking; it will then form a highly opalescent liquid. To the above mentioned portion 500—600 cc. $^n/_5$ sulphuric acid will be required, and commonly the more the older the eggs are.

After addition of the acid and vigorous stirring, the crystallisation will commonly begin in course of an hour or so; it is promoted by frequent stirring or shaking, as well as by addition of a little "inoculating" material (crystals with adherent mother liquor) from a previous crystallisation. When the crystallisation is well in operation, the mixture is left to stand at the ordinary temperature for a few (2—5) days, and repeatedly stirred. Then it is filtered without suction by ordinary filters, and for the sake of the washing the precipitate is distributed among several filters; for the portion here mentioned 5 filters (diameter 24 cm.) will do. Should the mother liquor, even if left to stand till the next day, not yet have been completely filtered off, it may be carefully removed with a pipette or decanted, as the precipitate will adhere rather firmly to the filter.

For the purpose of determining the composition of the washing liquid, mixtures are prepared in a series of test-tubes, each containing 10 cc. of saturated ammonium sulphate solution and different quantities of water; to each of these mixtures are added a few cubic centimeters of the filtrate which has run through. If now it appears that for instance the mixture "10 cc. $(NH_4)_2\ SO_4$-solution + 6 5 cc. water" as well as all mixtures richer in ammonium sulphate grow turbid by this addition of filtrate, while the mixture "10 cc. $(NH_4)_2\ SO_4$-solution + 7.0 cc. water" and all mixtures poorer in ammonium sulphate remain clear, then a mix-

ture of saturated ammonium sulphate solution and water in the ratio 10 cc. to 7 cc. is used as washing liquid. The filters are filled completely with this mixture without stirring up the precipitate, and then the washing liquid will gradually displace the mother liquor in the precipitate.

The next day whatever part of the washing liquid may not yet have run through is decanted from the precipitate, and the latter scraped off from the filters as well as possible into a spacious china dish by means of a porcelain spoon. The residue of precipitate left on the filters is dissolved in well-boiled water by rubbing with a spatula, the solution obtained in this way is mixed with the precipitate in the china dish, and boiled water added in sufficient quantity to completely dissolve the precipitate by careful and repeated stirring.

The solution thus obtained is poured into a cylinder glass and is caused to crystallise again by adding so much saturated ammonium sulphate solution that the amorphous, voluminous precipitate separated is just dissolved again by careful and continued stirring. By frequent stirring, and far more easily by the addition of a little inoculating material, the crystallisation will soon begin, and the method is now exactly the same as described in regard to the first crystallisation.

As will be mentioned later on, the egg-albumin crystallised out is already after three crystallisations practically free from ash, mucoid and conalbumin, but we have always, for safety's sake, crystallised six times, and the solutions obtained after the third and fifth crystallisations were filtered in order to free them from filter paper and denaturated albumin.

For the precipitation of the egg-globulin as well as for the first filtration and crystallisation, ordinary "pure" ammonium sulphate and ordinary filter paper were used; but in all subsequent operations the purest ammonium sulphate which we were able to procure was used (in order not to add ash to the egg-albumin), and we also used especially pure, washed filter paper (Nr. 590) from the firm of Schleicher & Schüll, Düren.

In order to be enabled to follow quantitatively the progressive purification of the egg-albumin through repeated crystallisation, we have made several series of experiments, a single one of which will here be more thoroughly described by way of example.

As initial material one liter of a solution of egg-white freed

from globulin in the way described above was used, the contents of ash and of coagulable and non-coagulable nitrogenous matter having been previously estimated. The egg-albumin in this solution was crystallised out three times, and the contents of ash and distribution of the nitrogen determined in the filtrates and washing liquids as well as in the final solution, obtained by dissolving the precipitate crystallised three times. All the vessels and filters used in the crystallisation and filtration were carefully washed, and the solutions thus obtained (they are marked "Residue") were also analysed. The results of these analyses are set forth in the Table 1 (see p. 16)[1]), which is referred to in the following separate sections for each of the impurities here dealt with: ash, mucoid and conalbumin.

b. Ash.

For the purpose of determining the amount of ash, the solution was evaporated, and the organic matter and ammonium salts were removed by heating, in an exactly weighed, spacious platinum dish, which was placed in a Heraeus muffle furnace electrically heated, in which the temperature could be very easily regulated both at temperatures below 100° during the evaporation and at higher temperatures during the further heating. For the purpose of avoiding impurities from the furnace material, the inside of the furnace was covered with platinum plate, so that the platinum dish during the process only came in contact with platina. In the furnace-funnel was placed a gaslight, which burned the inflammable, malodorous gases produced by the carbonization of the protein. As all the solutions used for ash-determination either contained plenty of ammonium sulphate, or before evaporation were mixed with some solid ammonium sulphate, the basic components of the ash were weighed mainly as sulphates. After the first weighing the ash was wetted with strong sulphuric acid, and after evaporation and a short heating it was weighed again.

From Table 1, columns 2 and 3, it appears that by far the greatest part of the ash is removed through the first crystallisation

[1]) From the lowest row of Table 1 it will be seen that while the whole amount of ash found is very near 100 % of the amount present, about 2 % is lacking in protein nitrogen. This is doubtless due to the fact that, in spite of a fairly quantitative mode of working, we have not succeeded in washing all the protein out of the large filters used in the filtration, and the figures for the protein nitrogen in the solutions marked "Residue" are therefore rather low.

TABLE 1.

Purification of Egg-albumin by Crystallisation.

	Amount of ash		Amount of nitrogen in *non-coagulable* compounds ("*Mucoid-nitrogen*")		Amount of nitrogen in *coagulable* proteins		*Coagulable*, but *non-crystallisable* proteins ("Conalbumin")						
							Mother liquor:			Contents of *crystallisable* egg-hydrate per 100 g. water in a mother liquor of the actual ammonium sulphate and hydrogen-ion concentration	Contents of *Conalbumin* per 100 g. water in mother-liquor	Protein in the whole of the mother liquor. Percentage of the total coagulable proteins of the initial material	
							P_H	contents pr. 100 g water of				*crystallisable* protein	*non-crystallisable* protein
	g.	percentage of the total amount of ash	mg.	percentage of total mucoid nitrogen	mg.	percentage of total nitrogen of coagulable proteins		Am_2SO_4	egg-hydrate				
								g.	g.	g.	g.		
Original solution..	5.122	100.00	894.1	100.00	6608.7	100.00	—	—	—	—	—	—	—
Filtrate I.....	4.545	88.73	803.8	89.90	1908.1	28.87	4.604	27.789	1.421	ca. 0.274	ca. 1.147	ca. 5.57	ca. 23.30
Washing liquid I.	0.486	9.49	57.8	6.46	177.2	2.68	—	—	—	—	—	—	—
Residue I.....	0.039	0.76	4.4	0.49	53.0	0.80	—	—	—	—	—	—	—
Filtrate II	0.022	0.43	13.4	1.50	311.0	4.71	4.652	28.479	0.292	ca. 0.195	ca. 0.097	ca. 3.15	ca. 1.56
Washing liquid II	0.008	0.16	—	—	10.7	0.16	—	—	—	—	—	—	—
Residue II.....	—	—	—	—	44.8	0.68	—	—	—	—	—	—	—
Filtrate III.....	0.008	0.16	0.0	0.00	193.5	2.93	4.699	28.365	0.234	ca. 0.223	ca. 0.011	ca. 2.79	ca. 0.14
Washing liquid III	0.006	0.12	—	—	2.3	0.03	—	—	—	—	—	—	—
Residue III.....	—	—	—	—	37.5	0.57	—	—	—	—	—	—	—
Final solution....	0.006	0.12	0.0	0.00	3741.0	56.61	—	—	—	—	—	—	—
Total..	5.120	99.97	879.4	98.35	6479.1	98.04	—	—	—	—	—	—	—

and washing; noticeable quantities of ash are, however, also found in Filtrate II, while the minimal quantities found in Filtrate III and in the final solution are, as we soon shall see, mainly due to the phosphorus contained in the egg-albumin itself. In fact, the qualitative examination of the ash samples gave the following result:

The ash from "the Original Solution", "Filtrate I", "Washing Liquid I" and "Residue I" behaved alike. It was found to be almost completely soluble in water, and the filtered solution showed a neutral reaction towards litmus, and contained plenty of sulphate, but no chloride. The solution further contained small but noticeable quantities of calcium salt, but only a trace of phosphate; nor did that part which was insoluble in water contain more than a trace of phosphate. The phosphoric acid of the genuine egg-white may be precipitated as calcium phosphate or as magnesium ammonium phosphate together with the globulin in ammoniacal liquid.

The aqueous extract of the ash from "Filtrate II" gave a definite reaction for sulphate, but only an exceedingly slight one for phosphate. Subsequent treatment of the platinum dish, however, which was spotted here and there with small gray stains, with warm quite weak nitric acid, yielded a solution which gave a slight reaction for calcium salt and a well-marked reaction for phosphoric acid.

The aqueous extract of the ash from "Filtrate III" gave a slight but definite reaction for sulphate, and a barely perceptible reaction for phosphate. In the nitric extract only a slight trace of calcium salt was found, but appreciable quantities of phosphoric acid.

Finally, the aqueous extract of the ash from the final solution gave only a barely perceptible reaction for sulphate, and no reaction for calcium salt, but did give a slight reaction for phosphoric acid. Upon the platinum dish several gray stains were noticed, which on treatment with warm, quite weak nitric acid, disappeared almost completely; the nitric extract did not contain calcium salt, but distinct quantities of phosphoric acid. There can therefore hardly be any doubt that the gray stains contain phosphorus, probably combined with platinum. In harmony with this result the weight of the platinum dish was found to decrease by a few milligrams for each determination of ash and subsequent

purification. This phosphorus doubtless does not originate in any impurity, but in the egg-albumin itself, which according to the analyses of Thomas B. Osborne and G. F. Campbell[1]) contains 0.12 % phosphorus. Paying regard to this fact, and moreover considering that the "ash" obtained from the final solution contained only an exceedingly slight trace of sulphate and no calcium salt, it seems pretty certain that the egg-albumin solution already after three crystallisations is practically free from ash[2]).

The greatest difficulty in the preparation of ash-free egg-albumin solutions is to procure sufficiently pure ammonium sulphate. Even the purest preparation in the market, furnished by the firm C. A. F. Kahlbaum, Berlin, and marked "Zur Analyse, mit Garantieschein", is not always sufficiently pure for this use. In 100 g. of such ammonium sulphate preparations we have often found 2—5 mg., on one occasion 9 mg. ash, which sometimes contained calcium sulphate and often plenty of phosphate. That the substance here in question is really phosphoric acid and not arsenic acid, as was rather to be expected, was shown by transformation of the yellow precipitate obtained with ammonium molybdate into magnesium ammonium phosphate and a subsequent examination by the test of Marsh. At our request, however, the firm C. A. F. Kahlbaum has with great willingness prepared an ammonium sulphate purified with special care, which in 100 g. contains still less than 1 mg. ash. It is only by the use of such a very pure ammonium sulphate that it is possible to prepare egg-albumin free from ash

[1]) Journ. Americ. Chem. Soc. **22**, 440 (1900).

[2]) In this connection we may mention another experiment, which was made with a solution of egg-albumin six times re crystallised and then, by dialysis, freed from ammonium sulphate. After adding equal portions of $^n/_1$ acetic acid and $^n/_1$ sodium acetate solution, the solution was coagulated, the precipitate filtered off, washed with hot water, and then boiled several times with absolute alcohol. The alcoholic extract was evaporated to dryness in a platinum dish on a water bath, leaving only a hardly visible residue, which after wetting with an alkaline solution of sodium nitrate was dried up and heated; in the residue no trace of phosphate could be shown. The egg-albumin coagulated and boiled with alcohol was in turn wetted with the sodium nitrate solution, and then dried up and heated until the organic matter was burned away. In the residue plenty of phosphate was found. This result speaks in favour of the view expressed by E. G. Willcock and W. B. Hardy (Proc. Cambridge Philos. Soc. **14**, 119 (1907), cited after Chem. Zentralbl. 1907 II, 821), that the phosphorus forms an integral part of the protein molecule.

(Concerning the possible contents of "surplus" sulphuric acid or ammonia in the ammonium sulphate, we refer the reader to Paper II).

c. Mucoid.

As above mentioned, we use the name *Mucoid* as a common designation for all nitrogenous substances contained in the egg-white which do not coagulate by heating, and therefore the determination of the mucoid nitrogen is made after removing the ammonia from the filtrate of the coagulated proteins, by determination of the total nitrogen in the residue according to Kjeldahl's method. Concerning the detail of this method we refer to Section D, Methods for Nitrogen Determination; here only a single point need be mentioned, which is of no consequence in the analysis of fairly pure egg-albumin solutions, but is of great significance in the analysis of such solutions in which most of the components of the genuine egg-white are still present.

In an earlier paper[1]) mention was made of the fact that the amount of nitrogen which remains in the solution by heating a globulin-free but genuine egg-white solution depends on several factors, namely — besides the hydrogen-ion concentration and the contents of salt (which always are of more or less consequence for the coagulation of the protein) — on the duration of the heating and the amount of the precipitate coagulated out. If we now — as in the purification experiments described in this paper — wish to compare the amounts of non-coagulable proteins in a solution used as initial material and in the filtrate obtained by the crystallisation, which is poorer in egg-white but richer in ammonium sulphate, we must take care, before coagulation, to dilute the sample of the initial material with a proper ammonium sulphate solution to such an extent that the coagulation in both samples will take place under as identical circumstances as possible. We have of course paid regard to this point in our experiments, the results of which are set forth in Table 1 (p. 16).

It is seen from the figures given in the fourth and fifth columns that the purification from the mucoid upon the whole goes on in quite the same way and as easily as the purification from the ash. By far the larger part of the mucoid is removed by

[1]) S. P. L. Sörensen and E. Jürgensen: Comptes-rendus du Laboratoire de Carlsberg **10**, 1 1910).

the first crystallisation, and after three crystallisations the quantity of mucoid is so minimal that it is not possible to detect it in this way.

d. Conalbumin.

While the determination of the amount of ash and of the contents of non-coagulable nitrogenous matters can thus be comparatively easily made with due exactness, this is not the case with the determination of the amount of coagulable but not crystallisable egg-albumin, which, as before mentioned, is comprised under the common name *Conalbumin*. The fact is that it is not possible by crystallisation under such circumstances (hydrogen-ion and ammonium sulphate concentration) as used in the common purification of the egg-albumin through repeated crystallisations, to remove the whole amount of crystallisable egg-albumin present, and therefore the mother liquor will always contain both non-crystallisable and crystallisable egg-albumin. The amount of the latter will, as mentioned more fully in Paper IV, depend on a variety of circumstances, especially the hydrogen-ion concentration of the mother liquor and its content of ammonium sulphate; further, on the temperature and time of crystallisation, but probably also on the impurities in the mother liquor and the frequency with which the mixture has been shaken or stirred during crystallisation. Nevertheless, however, we have believed to be able to form an idea as to whether repeated crystallisations give an effective purification with regard to the conalbumin present in the genuine egg-white solution too, and to this end we have used the following method:

The mother liquor to be examined was analysed, and the amount of ammonium sulphate and egg-hydrate per 100 g. water was calculated, using the factor 7.86[1]) for the whole amount of protein nitrogen present. By means of the results obtained by the investigations as to equilibrium between the egg-albumin crystallised out and the surrounding mother liquor (*vide* Paper IV) it was then estimated how much crystallisable egg-hydrate per 100 g. water there would be in the mother liquor under the existing circumstances (ammonium sulphate and hydrogen-ion concentration, time of crystallisation, and temperature), taking it for

[1]) For determination of the numeric quantity representing the factor by which the weight of the protein nitrogen has to be multiplied to give the weight of the corresponding amount of egg-hydrate, we refer to Papers III and V.

granted — which probably is not quite right — that other circumstances than those here mentioned, for instance possible content of conalbumin, did not influence the velocity and completeness of the crystallisation. By comparing the content of crystallisable egg-hydrate "calculated" in this way with the content of egg-hydrate found, we were able to estimate the content of non-crystallisable egg-hydrate. As an illustration may be cited the examination of Filtrate I from the series of experiments given in Table 1 (p. 16). The analysis of this filtrate showed that

100 g. filtrate contained:	
0.331 g. ash	0.331 g. ash
4.524 - ammonia-N corresponding to	21.337 - $(NH_4)_2SO_4$
0.1388 - "coag. egg-N"	1.091 - coag. egg-hydrate
0.0584 - "non-coag. egg-N"	0.459 - mucoid
	76.782 - water
	100.000

Per 100 g. water was then found { 27.789 g. $(NH_4)_2SO_4$; 1.421 - coag. egg-hydrate

The time of crystallisation was 4 days at indoor-temperature (18°), and the hydrogen-ion concentration in Filtrate I corresponded with $p_H = 4.604$.

From the graph given in Paper IV of the experiments relating to the influence of the ammonium sulphate concentration on the equilibrium between the precipitate crystallised out and the surrounding mother liquor, it will now be seen that the latter after a lapse of four days at 18° will contain 0.422 g. egg-hydrate per 100 g. water, if the content of ammonium sulphate in the mother liquor per 100 g. water is 27.789 g. and the hydrogen-ion concentration corresponds with $p_H =$ about 4.85.

However, the hydrogen-ion concentration in Filtrate I does not correspond with $p_H = 4.85$, but with $p_H = 4.604$, at which hydrogen-ion concentration the crystallisation — under otherwise equal circumstances — is more complete than it is at $p_H = 4.85$. The graph (the middle curve in the topmost bunch of curves corresponds with a time of crystallisation of five days at 18°) given in Paper IV on the influence exerted by the hydrogen-ion concentration on the equilibrium between the precipitate crystallised out and the surrounding mother liquor, shows that a mother liquor containing 25.947 g. $(NH_4)_2SO_4$ per 100 g. water

if $p_H \cdot = 4.85$, contains 0.640 g. egg-hydrate per 100 g. water
and if $p_H \cdot = 4.604$, — 0.412 - — - — - —

The last-mentioned mother liquor will thus contain only 64,4 % of the amount of egg-hydrate in the first mentioned.

By using the middle curve in the lowest bunch of curves, which corresponds with a time of crystallisation of five days at 18°, it will further be seen that a mother liquor containing 27,121 g. $(NH_4)_2SO_4$ per 100 g. water

at $p_H \cdot = 4.85$ contains 0.326 g. egg-hydrate per 100 g. water
and at $p_H \cdot = 4.604$ — 0.212 - — - — - —

Thus, also at this ammonium sulphate concentration, the amount of egg-hydrate will at $p_H \cdot = 4.604$ be a similar part to that above (65.0 %) of the amount of egg-hydrate at $p_H \cdot = 4.85$.

Without committing any essential error we may then take it for granted that the case will be quite similar at the ammonium sulphate concentration 27.789 g. per 100 g. water; consequently, as the amount of egg-hydrate in a mother liquor with this ammonium sulphate concentration and with $p_H \cdot = 4.85$ as before mentioned (see p. 21) will be 0.422 g., it will with $p_H \cdot = 4.604$ be about $0.422 \times 0\,65 =$ about 0 274 g.

The result of this estimate is then, that a mother liquor will contain about 0.274 g. crystallisable egg-hydrate if, like Filtrate I, it is obtained by crystallisation in four days at 18°, and contains 27.789 g. ammonium sulphate per 100 g. water, while having a hydrogen-ion concentration corresponding with $p_H \cdot = 4.604$. Now, as the whole amount of egg-hydrate found is 1.421 g. per 100 g. water, the content of non-crystallisable egg-hydrate will be about 1.147 g. per 100 g. water.

By a quite analogous mode of reasoning we have found, that Filtrate II and Filtrate III will contain, respectively, about 0.195 g. and about 0.223 g. crystallisable egg-hydrate per 100 g. water, and as seen from the figures in Table 1, columns 12 and 14, this signifies that Filtrate II still contains a slight, but appreciable amount of conalbumin, while the amount of this substance in Filtrate III is so insignificant that it will scarcely show up by the method under consideration.

The case is thus the same with the conalbumin as with the contents of ash and mucoid in the genuine egg-albumin solution:

the main part disappears already in the first re-crystallisation with subsequent washing, and after three re-crystallisations there will probably be left only a trace of conalbumin.

In this connection we may draw attention to the experiment described on p. 37 as also to those mentioned in Paper IV under the section treating of the influence exerted by the protein concentration upon the equilibrium; these experiments suggest that in aqueous solution, the crystallisable egg-albumin may possibly in course of time be transformed into non-crystallisable, but completely coagulable albumin.

For the sake of completeness it may finally be added that we have made several series of experiments of quite a similar kind to that referred to in Table 1, though under other experimental conditions, for example crystallisation at ice-safe temperature or crystallisation with addition of a small dose of ammonia and succeeding addition of the equivalent amount of sulphuric acid, or conversely. As an example of the latter mode of crystallisation we may only mention the following: To 550 cc of a solution of egg-albumin once crystallised out and washed 200 cc. $^{n}/_{1}$ sulphuric acid and 200 cc. saturated ammonium sulphate solution were added; after being left to stand for half an hour the solution received an addition of 20 cc. $^{n}/_{1}$ ammonia and, subsequently, a saturated solution of ammonium sulphate to incipient permanent turbidity (some 150 cc. were found to be required). Our intention in doing so was, of course, to try if any basic substances that might be combined with the egg-albumin could be more easily removed by such a preliminary treatment with acid than by the usual method; the results of all our experiments corresponded, however, entirely with those set forth in Table 1.

We think we are justified in concluding from these experiments, that *the purification of egg-albumin from ash, mucoid and conalbumin does not offer any difficulty*. It is true that a closer examination of all the numerical results seems to indicate that the components of the ash are removed somewhat more easily than the mucoid, and this more easily than the conalbumin; but it may, however, be supposed that *none of these impurities*

is found in appreciable quantity after three crystallisations with the usual washings. As the whole of our initial material in the experiments described in the following papers was obtained from egg-albumin which had been crystallised at least six times. we feel justified in considering it as being free from the impurities in question.

B. Purification of Egg-albumin from Ammonium sulphate by Dialysis.

After purifying the egg-albumin by crystallisation, the next thing to be done was to remove the ammonium sulphate present as completely as possible, an operation which, of course, had to be executed by dialysis. It now turned out to be impossible by simple dialysis with pure water to remove every trace of the components of the ammonium sulphate, especially of the ammonia. This is not to be wondered at, if we consider that the egg-albumin, being an amphoteric compound, is able to combine with acid as well as basic substances, and especially with the latter, because egg-albumin is of a more pronounced acid than basic character. This being so, we have found it suitable to conduct the dialysis in such a way that all the sulphuric acid is removed, which can easily be done by adding, at proper times and at proper intervals, a little ammonia to the egg-albumin solution, by which means sulphuric acid still present combined with the egg-albumin is transformed into ammonium sulphate, which dialyses away. After the sulphuric acid has been completely removed, it is of course not possible, by dialysis, to completely free the egg-albumin solution from ammonia. The fact is that the egg-albumin, acting as an acid, combines with ammonia to form ammonium salt, and only the excess of ammonia readily dialyses away; later on it is only the ammonia released by the hydrolysis of the ammonium salt that is able to dialyse, and the hydrolysis will decrease more and more in the course of dialysis. Accordingly, as it is possible to determine small amounts of ammonia with a fair degree of exactness, we have ultimately adopted the method of dialysing which we are now going to describe: —

The greatest part of the ammonium sulphate is removed by dialysis against well-boiled distilled water in the course of about a week; then the egg-albumin solution is mixed with $^{n}/_{1}$ ammonia till the reaction towards litmus grows slightly but distinctly alkaline, and then the dialysis is continued against boiled water for about a week. To prevent the egg-albumin solution from being diluted in the course of dialysis, this operation is effected in an apparatus which permits the establishment of such a diminished pressure on the "outer liquid" (the water) that the osmotic pressure of the "inner liquid" (the egg-albumin solution) will be compensated. The treatment with $^{n}/_{1}$ ammonia is repeated once and, if necessary, twice, the sulphuric acid not being considered to have been completely removed until, by the test for sulphuric acid described later on (p. 32), no sulphuric acid can be detected either in the last dialysate before the addition of ammonia or in the first dialysate after addition of ammonia (compare p. 34). After the last addition of ammonia, the dialysis is still carried on for about a week against well-boiled water; then the egg-albumin solution is filtered and analysed. By this analysis the contents of protein nitrogen and ammonia nitrogen are determined. If now we add the quantity of sulphuric acid (or some other acid) equivalent to the amount of ammonia found, we have a *well-defined egg-albumin solution whose amount of protein nitrogen is known, and which besides egg-albumin contains only a slight and known amount of ammonium sulphate (or another ammonium salt), but neither surplus acid nor ammonia.* From such a solution it is of course possible by adding proper quantities of acids, salts or ammonia, to prepare egg-albumin solutions possessing widely different and yet completely known total compositions. As for the way in which the substances thus added are distributed among the two phases of the egg-albumin solution, this is another question, which we are not concerned with in the present paper (see Paper II and V).

In the following chapter we are now to describe more thoroughly the apparatus employed for the dialysis, together with different details concerning the execution and whole course of the dialysis.

a. The Apparatus of Dialysis.

The essential part of the dialysing apparatus is the dialysing cell, which is shown in Fig. 1. The cell consists of a bottle provided with two tubus (A and B). A hollow stopper (D), the upper part of which is funnel-shaped, is made exactly to fit the ground neck (C) of the bottle; the funnel is covered over by a small glass shade. The lower, slightly conical part of the stopper supports a collodion cap (E) formed like a test tube; the mode of preparation of this cap will be described later on. If the grinding has been carefully done, and if the cap fits the stopper, it is possible by a slight pressure on the stopper — and, if necessary, by subsequent covering with a little pure vaseline — to bring about a perfectly air-tight closing.

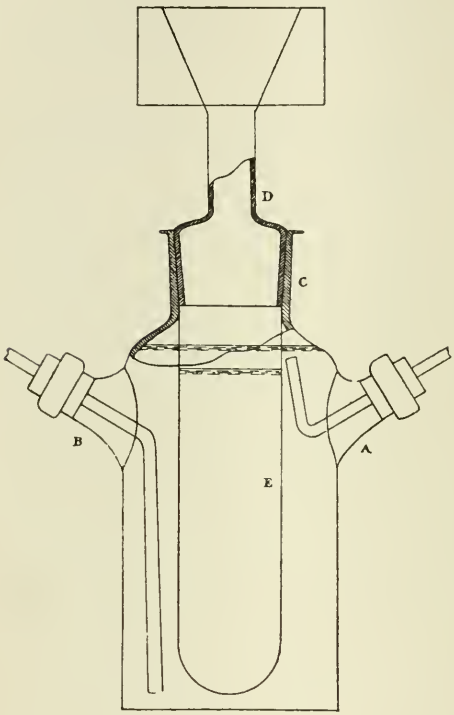

Fig. 1.

When the cell is to be used, the egg-albumin solution to be subjected to dialysis, which is well mixed with an abundant excess of toluol, is poured through the funnel into the cap, while well-boiled distilled water is introduced through B, after which the cell is placed in the dialysis apparatus.

The latter, of which Fig. 2 gives a sketch, consists of six cells placed one beside another, surrounded by crushed ice in a zinc box, which is placed in an ice-safe. Tubus B of each of the six cells is (by means of an indiarubber tube provided with a pinch-cock) connected with the glass tube F leading to the reservoir G, which is placed in the ice-safe and in which the boiled water set apart for the change of the outer liquid is kept

and cooled. By tubes penetrating the ceiling of the ice-safe, G can be filled from a reservoir placed upon the safe. An india-rubber tube provided with a pinch-cock connects tubus A with the bottle H, which serves for collecting the outer liquid used. Each cell has its own collecting bottle, and these six bottles are (through indiarubber tubes provided with pinch-cocks) connected

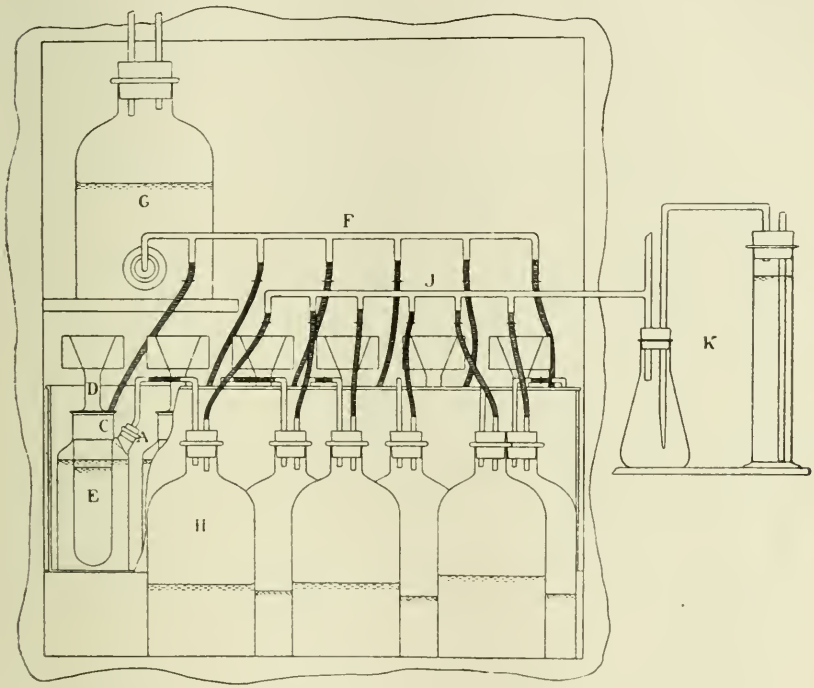

Fig. 2.

with the common tube J, which by a T-tube outside the ice-safe leads to the pressure regulator (K). and also to a water air-pump with accessory manometer (not shown in the Figure).

When the dialysis has to begin and the cells as described above have been filled and put in their places, the suction pump is set going, only the pinch-cocks between the tube J and the bottles H being opened. When, after some time, the pumping out has advanced so far that the regulator, which we have usually adjusted for a diminished pressure of 24—25 cm. mercury, begins to act, the pinch-cocks between the bottles H and the cells are cautiously opened, so that the diminished pressure is made to

act also upon the outer liquid in the cells, the osmotic pressure of the inner liquid being thus compensated. The apparatus may then be left to itself; the outer liquid must, however, at proper intervals be changed, which is done simply by cautiously opening the pinch-cock between the tube F and the cell concerned. Owing to the present diminished pressure, the outer liquid will then be sucked from the cell into H and replaced by fresh outer liquid from the reservoir G, which being graduated enables the operator to ascertain how much water has been used. When the bottles H are to be emptied, all the pinch-cocks between these bottles and the cells are closed so as to maintain the diminished pressure in the cells, the pinch-cocks not being opened again till the diminished pressure in the emptied bottles H has become re-established.

The dimensions we have chosen are the following: Each collodion cap will contain some 200 cc., each cell 600—700 cc. outer liquid, H will hold 4 l. and G 7 l. The outer liquid is changed three times in twenty-four hours, and at each change 1 l. water runs through each cell.

Preparation of the Collodion Caps. The method employed by us for the preparation of the collodion caps was in the main elaborated by Mr. J. A. Christiansen. It differs in a few points from current methods of preparing collodion membranes[1]).

It is to be noted that only certain definite sorts of collodion prepared with this object in view will answer the purpose. We formerly used C. A. F. Kahlbaum's "Kollodium zur Herstellung von Membranen für Dialyse"; but in the last year or so this preparation has turned out to be rather unsatisfactory. We therefore proceeded to purify it by filtration through filter paper, evaporation in a large dish by the aid of a hot-air blower till the ether and the greater part of the alcohol had been evaporated, when the remainder was carefully and repeatedly kneaded and rubbed with pure distilled water. After standing with water till the next day, the washed collodion cotton was filtered off and then, after drying in the air, packed up in filter-paper and dried to constant weight by means of the hot-air blower. About 30 g. of the com-

[1]) Concerning the earlier literature we may refer the reader to Wo. Ostwald: Grundriss der Kolloidchemie, Zweite Auflage, 1911, p. 12.

pletely dry collodion cotton was, by repeated shaking in the course of some days, dissolved in a mixture of 750 cc. absolute alcohol and 250 cc. anhydrous ether. The solution obtained in this way has in every case proved to be excellently fit for use, whereas solutions containing a larger proportion of ether sometimes caused some trouble, probably because the evaporation of the ether during the preparation of the cap was so brisk that, on account of the cooling, water was condensed on the cap, which then assumes a clouded appearance and acquires a too great permeability.

As mould for the membrane we use in the preparation a *thick*-walled test-tube washed outside with soap and then well wiped. It is provided with a little hole in the bottom (abt. 2 mm. in diameter)[1]), and is fitted (not hermetically) on to a cork-stopper attached to a horizontal shaft, which can be set rotating slowly (40—60 revolutions per minute) by a motor. The bearing of this shaft is so fastened as to permit a turning out of the horizontal position into a vertical plane.

To begin the preparation, the shaft is first placed in a somewhat inclined position (20^0—30^0); collodion is then poured on the bottom of the rotating tube, beginning a few cm. from the end. This is done partly in order to close the bottom hole in the test-tube, partly in order to strengthen the lower part of the cap. A few seconds after pouring on, the rotation is stopped for a few moments to let the superfluous collodion run off into a bottle with a funnel. After rotation for 6—8 min. the first real application is effected, while shaft and tube are constantly rotating in a slightly inclined position. The collodion is poured in a thin jet over the test-tube from the upper to the lower part, and the rotation is stopped a few seconds to let the superfluous collodion run off. Now the shaft is turned back to the horizontal position, and the rotation is continued for 6—8 minutes, at the end of which the pouring on is repeated in the same way as the first time.

We generally use four such applications, and, in addition to make the upper edge of the cap stronger, the latter is given an extra pouring on while rotating in the *horizontal* position.

[1]) The test-tube must never be wider below than at the top, and it must correspond exactly with the stopper D (see Fig. 1), so that the outside diameter is, as far as possible, equal to the diameter of the stopper D at the middle of the grinding; we usually employed test-tubes of 240 × 44 mm.

After the last application, the cap is dried for $1^1/_2$—2 hours in the air, the rotation in *horizontal* position being continued in every case for the first half hour. With a sharp pen-knife a cut is made all round the membrane near the upper end of the tube, which is then placed under water, in order to replace by water the alcohol left in the cap after drying in the air. After a few hours an air-bubble can be introduced at the upper edge of the cap, between the membrane and the glass. By cautious rubbing under a dripping water tap it is possible to pass this air-bubble round the test-tube and thus to loosen the membrane and draw it off, the hole in the bottom of the test-tube permitting the water in the tube to fill the space between the glass and the cap.

The cap is now ready for use, and can — without materially altering its permeability — be kept in water (saturated with toluol) or in a salt solution for an unlimited length of time; it must never get dry, however, as its permeability would thereby be considerably impaired.

When the cap is to be placed in the dialysing-cell on the stopper D (see Fig. 1), it is first cautiously enlarged at the aperture — just as the finger of a glove is enlarged — either by means of a finger or better by means of a proper form, after which the cap is thrust on the lower ground part of the stopper D. When the cap has been raised a few millimeters, it is held under a dripping water tap, so as to wet both the stopper and the cap, which latter must point downward and forward. Then the cap is rubbed with the thumb directed towards the operator, while turning the whole a little at every "rub". In this way the cap may little by little be drawn up to a height of 1—2 cm. over the lower edge of the grinding; but the fastening of the cap is a rather difficult operation, which calls for great caution and practice, and the cap must be of good quality or it will burst.

When the cap is fastened, the stopper with the cap is filled with water and put in its place in the cell[1]), which is then tested before use to see if the apparatus is tight and the cap sufficiently

[1]) Sometimes this can be done without difficulty; but the diameter of the cap is usually so large that it is necessary to use the following method. As above mentioned, the cap is filled with water and the lower part of it is placed in the mouth of the cell. Supporting the cap with the left hand, the operator lifts the whole a very little way from the table and then lets it fall. When this has been repeated some few times, the cap filled with water will, on account of its weight, glide into place.

strong, and also if the permeability of the cap is as required. The first examination is made simply by filling the cell with water both inside and outside the cap, closing the tube through Tubus A (see Fig. 1) and then through Tubus B cautiously and gradually producing a somewhat greater vacuum than that at which the cap is to be used later on. If the apparatus is found to leak, air being sucked in at the stopper D, this deficiency may usually be remedied by tightening with a little vaseline; if the cap is leaky so that water or air is sucked through, it must be rejected; if it is too weak, it will burst during the test.

The permeability of the cap depends partly on the thickness of the wall, partly on the time of drying between each pouring on in the preparation, and more especially it depends on the time of drying reckoning from the last pouring on to the immersion in water, the permeability being the less the longer the time of drying has been. The permeability is tested by placing an egg-albumin solution containing ammonium sulphate as inner liquid and pure water as outer liquid in the cell and then leaving the whole to stand for twenty-four hours under that diminished pressure at which the cell is to be used. The next day the outer liquid is completely replaced by pure water, and the following day this operation is repeated, after which the outer liquids from the three days' dialysis are examined separately. The permeability is right if the ammonium sulphate has gone through so readily that the third dialysate is found to contain only comparatively small amounts of sulphuric acid, while the egg-albumin can only be shown to exist as a trace, if at all, in any of the dialysates. The test for the egg-albumin is made by adding 10 cc. $^{n}/_{1}$ acetic acid and 10 cc. $^{n}/_{1}$ sodium acetate solution to 100 cc. of the dialysate and heating on a water-bath for half an hour. If more than a quite insignificant precipitate is thrown down by this test, the cap must be rejected. The test is a very delicate one, it having been shown by control experiments that even so small amounts of egg-albumin as those corresponding to 0.1—0.2 mg. protein-nitrogen produce a slight opalescence and permanent foam It sometimes happens that a cap in the first day's dialysis appears to be slightly permeable to egg-albumin, and that the permeability is lost in the course of the test; such a cap is of course perfectly fit for use. A well prepared dialysing-cell may be used for years, provided that it is carefully purified every

time it has been used, and that it is preserved with the help of toluol-containing water or saturated ammonium sulphate solution, in which it will remain sterile.

The dialysis apparatus described above may be advantageously used not only in the experiments here mentioned, but in any dialysis in which it is desirable to avoid a too strong dilution of the solution to be dialysed. As it is easy, by modifying the method of preparation of the caps, to alter their permeability, the apparatus admits of being used in the dialysis of widely different solutions, the permeability of the cap being in each particular case adjusted to the use for which it is intended. Thus we have used the apparatus for instance in the purification of several enzyme solutions and obtained not only a dialysing away of salts, amino acids etc., but also a considerable concentration of the enzyme solution.

b. The Test for Sulphuric Acid.

Test in the Dialysate. 2 cc. $^{n}/_{1}$ sodium acetate solution and 2 cc. $^{n}/_{1}$ acetic acid are added to 1 l. dialysate, which is then evaporated *in vacuo* in a carefully purified flask fitted with a boiled cork stopper and well purified glass tubes. The air which during the evaporation is passing through the capillary tube is first sent through a small washing bottle containing sodium hydroxide solution in order to be freed from sulphurous acid. After evaporating down to a few cc., the liquid is filtered through a small well washed filter into a clean test tube, and the flask and filter are washed with pure water till the total volume of the filtrate amounts to about 15 cc. 5 drops of a 2n barium chloride solution are then added to the filtrate, which must be carefully shaken, and any turbidity that may possibly have been produced is compared with the turbidity observable in a series of control samples made at the same time, each containing 2 cc. $^{n}/_{1}$ sodium acetate solution + 2 cc. $^{n}/_{1}$ acetic acid + a cc. $^{n}/_{100}$ sulphuric acid + water completing up to 15 cc., and, finally, 5 drops of a barium chloride solution, where a ranges from 0 to 2.

Test in the Egg-albumin solution. 50 cc. egg-albumin solution are mixed with 200—300 cc. water, and subsequently, while

being well shaken, with 2 cc. $^{n}/_{1}$ sodium hydroxide solution. After standing at the ordinary temperature for half an hour, the mixture receives an addition of 4 cc. $^{n}/_{1}$ acetic acid and is subsequently coagulated, while being carefully and repeatedly shaken, on a boiling water-bath for $^{3}/_{4}$ hour. The precipitate is filtered off on a well washed filter and washed several times with water, which done the filtrate and washing water are evaporated *in vacuo* and treated in the manner described above.

Control experiments made partly with pure water, partly with an egg-albumin solution which through a prolonged dialysis combined with repeated additions of ammonia had been freed completely from sulphuric acid, have shown that the limit of sensibility of the method here described is at 0.2—0.3 cc. $^{n}/_{100}$ sulphuric acid, while smaller amounts of sulphuric acid cannot be detected with certainty. We have further established that with amounts of sulphuric acid ranging between 0.2 and 2 cc. $^{n}/_{100}$ it is possible to estimate quantitatively the amount of sulphuric acid present with a fair degree of exactness by means of the above mentioned comparison between the sample under examination and control samples with known contents of acid. In the presence of 2—4 cc. $^{n}/_{100}$ sulphuric acid the comparison is less certain, and with greater amounts the method is hardly applicable.

We conclude from these control experiments that, if a dialysate or an egg-albumin solution on being tested for sulphuric acid has given a clear solution or a turbidity less than that shown by the control sample with 0.3 cc. $^{n}/_{100}$ sulphuric acid, it is either free from this acid or contains at most 0.3 cc. $^{n}/_{100}$ in the quantity used for the test.

c. The Course of the Dialysis.

In order to throw some light upon the process of dialysis, we will here describe in greater detail one of those experiments the whole course of which we have followed through quantitative determinations made on the dialysates.

610 g. of an egg-albumin solution containing egg-albumin corresponding to 12.6 g. protein nitrogen, and ammonium sulphate corresponding to 16.204 g. ammonia nitrogen, were distributed, on the 11th of September 1914, into the six cells of the

apparatus, the caps of which were thereby half filled; the egg-albumin solution was saturated with toluol, and the surface of the inner liquid was covered with the same substance. The whole of the first dialysate, *viz.* the dialysates from the first three changings (Sept. 11th—12th), weighed 19130 g. The amount of ammonia in three weighed samples of about 30 g. each was determined as exactly as possible; the whole of the first dialysate contained 14.912 g. ammonia nitrogen, or 92.03 % of the total amount. The second, third and fourth dialysates were treated in the same manner; the following dialysates were measured, and from measured portions of these the ammonia was distilled off and determined after the method of Nessler (see p. 55). The results were as follows:

		Weight or volume of the dialysate	Amounts of ammonia nitrogen	
			g.	Percentage of the total-ammonia nitrogen
First dialysate	(Sept. 11th—12th)	19130 g.	14.912	92.03
Second „	(„ 12th—13th)	17370 „	1.015	6.26
Third „	(„ 13th—14th)	17850 „	0.137	0.85
Fourth „	(„ 14th—15th)	17700 „	0.044	0.27
Fifth „	(„ 15th—16th)	17730 cc.	0.0226	0.14
Sixth „	(„ 16th—17th)	17770 „	0.0111	0.07
Seventh „	(„ 17th—18th)	18140 „	0.0096	0.06
Eighth „	(„ 18th—19th)	16220 „	0.0050	0.03
		Sum	16.1563	99.71 .

In the eighth dialysate the amount of sulphuric acid was also determined. with the result that 1 l. dialysate contained 1.2 cc. $^{n}/_{100}$ sulphuric acid.

On the 19th of Sept. the apparatus was dismounted, the lower pressure being maintained in all the cells; subsequently the contents of all the caps were emptied into a bottle by means of a rubber tube (working as a siphon and boiled several times in water). To the egg-albumin solution. ammonia was added by little and little, while shaking vigorously, to slight but distinct alkaline reaction towards litmus (23.2 cc. $^{n}/_{1}$ ammonia was consumed). After repeated shaking, the egg-albumin solution was again filled into the caps, the funnels were washed with some boiled water, a little toluol was added, and the dialysis was continued.

The next (the ninth) dialysate was found to contain, not

only more ammonia than the eighth dialysate — as was to be expected —, but also a larger quantity of sulphuric acid, namely, in all, 0.0154 g. ammonia nitrogen, and in 1 l. dialysate about 6 cc. $^{n}/_{100}$ sulphuric acid; but in the following (tenth) dialysate the total amount of ammonia nitrogen had already gone down to 0.0066 g., and 1 l. dialysate showed no more than 0.6 cc. $^{n}/_{100}$ sulphuric acid.

The dialysis was now continued till the 30th of Sept., during which time the amount of ammonia in the dialysate was seen to decrease by slow degrees from 0.0066 g. to 0.0041 g. ammonia nitrogen. Sulphuric acid could be detected in the dialysates of the first, but not in those of the last days.

On the 30th of Sept. the apparatus was again taken to pieces, and ammonia was, as above, added till the reaction of the egg-albumin solution became slightly but distinctly alkaline towards litmus (5 cc. $^{n}/_{1}$ ammonia was consumed), and then the dialysis was continued till $^{7}/_{10}$. The ammonia nitrogen in the dialysates of this period decreased gradually from 0.0126 g. to 0.0052 g. In 1 l. of the dialysate of the first day ($^{30}/_{9}$—$^{1}/_{10}$) 0.4 cc. $^{n}/_{100}$ sulphuric acid was found; in the later dialysates it was not possible to detect the presence of sulphuric acid.

On the $^{7}/_{10}$ the dialysis was stopped and the caps were emptied; in spite of the vacuum used (25 cm. mercury) the volume of the egg-albumin solution was increased considerably, the inner liquid filling the caps and rising up into the funnels; the volume of the whole amounted to a little more than 1400 cc. *Sulphuric acid could not be detected in 50 cc. of the egg-albumin solution.* 100 cc. of the solution contained 837.6 mg. protein nitrogen and 23.0 mg. ammonia nitrogen.

From this typical example of the course of a dialysis it results that by far the greater part of the ammonium sulphate dialyses out very rapidly. On the other hand it also appears that, even by a dialysis of long duration, it will be very difficult, if possible at all, to remove the last trace of the two components of the ammonia sulphate. It is further seen that the addition of ammonia to the egg-albumin solution tends to increase the amount of sulphuric acid in the dialysate (by the first addition of ammonia from 1.2 to 6 cc. $^{n}/_{100}$ sulphuric acid, by the last from 0 to 0.4 cc. $^{n}/_{100}$ sulphuric acid). As the addition of ammonia leads to a considerable increase in the osmotic pressure of the

egg-albumin solution, a vacuum of 25 cm. mercury will not be sufficient to prevent the inner liquid being somewhat diluted by absorption of water. This inconvenience can, however, be partly remedied by continuing (without further addition of ammonia) the dialysis still longer than in the example given above; the ammonia will then gradually, but in smaller and smaller amounts, dialyse away, and consequently the osmotic pressure of the egg-albumin solution will be diminished and a corresponding concentration of the inner liquid will take place; but a further dialysis, extending over several weeks, will then be required.

d. Does Egg-albumin undergo any, and what, Alteration in the Course of Dialysis?

Amongst the factors which are usually and justly mentioned as bearing upon the properties of colloidal solutions, are the age and antecedents of the solution. Seeing now that such investigations as those dealt with in this and the following papers would be of slight use if the properties of the initial material (the egg-albumin solution) were altered during the keeping of the solution, our constant endeavour has been to prove that our egg-albumin solutions do not undergo any alteration at all when they are kept and treated with the necessary care. The result of these researches is that the main source of the alterations undergone by egg-albumin solutions under the conditions chosen is the action of micro-organisms. If this action is obviated, by an abundant use of toluol, by often repeated shaking and by preserving the solution in ice in an ice-safe, it is possible to *keep the original and specific properties of an egg-albumin solution unaltered for months.* Neither will the egg-albumin be altered in course of dialysis, when the precautions just mentioned are used. This is best proved — as mentioned more fully in Paper V — by the fact that the osmotic pressure of a dialysed egg-albumin solution to which ammonium sulphate has been added is quite the same, under otherwise equal circumstances, as that of a not dialysed egg-albumin solution with the same amount of ammonium sulphate. And it is especially through the meaurement of the osmotic pressure that an incipient minimal splitting up may be observed with the highest degree of distinctness.

We have further inquired into the question as to whether one of the most characteristic properties of egg-albumin, the faculty of being separated in crystalline form by addition of ammonium sulphate, undergoes any alteration during dialysis. We find that an egg-albumin solution dialysed in the normal way by addition of ammonium sulphate and a little inoculating material crystallises out, practically, as completely as a non-dialysed egg-albumin solution. Thus, a normal dialysis does not give rise to any appreciable formation of non-crystallisable albumin. If, however, an egg-albumin solution, in consequence of improper treatment — say by standing a few days at the ordinary temperature without sufficient admixture of ammonium sulphate or of toluol — happens to be infected by bacteria of putrefaction, which will always make their presence felt by the smell after removal of the toluol by means of a current of hydrogen, then an essential part of the *crystallisable* egg-albumin will be transformed into *non-crystallisable* albumin, even if the decomposition has not advanced so far that more than quite insignificant amounts of protein are found to be present as *non-coagulable* compounds. Hence, the transformation of crystallisable into non-crystallisable albumin would seem to represent the first phase of the splitting up of the egg-albumin[1]).

We may finally mention a few experiments illustrative of the circumstances here referred to.

120 cc. of a dialysed egg-albumin solution, which had been filtered from the excess of toluol, but still smelled strongly of this substance, and which contained in total 4.1752 g. protein nitrogen, was precipitated by 66.5 cc. saturated ammonium sulphate solution and 5 drops of inoculating material. The crystallisation took a normal course, and under the microscope the crystals presented their usual appearance. After standing eight days at the ordinary temperature while being well shaken, the crystals were filtered off and the filtrate was analysed. The filtrate, which had a hydrogen-ion concentration corresponding with $p_H = 4.86$, was found to contain per

$$100 \text{ g. water} \begin{cases} 0.667 \text{ g. egg-hydrate} \\ 27.122 \text{ - ammonium sulphate.} \end{cases}$$

From the investigations reported in Paper IV (compare also p. 20) a filtrate obtained by eight days' crystallisation at the ordinary tempe-

[1]) The idea here suggests itself that the conalbumin in the genuine egg-white is to be regarded as a product of transformation of the crystallisable egg-albumin, which is often termed "ovalbumin". This conjecture agrees with the well-known fact that new-laid eggs give a higher yield of crystallised egg-albumin than eggs which have been kept for a length of time.

rature containing 27.122 g. ammonium sulphate per 100 g. water and having a hydrogen-ion concentration corresponding with $p_H = 4.86$, may be estimated to contain, approximately, 0.570 g. crystallisable egg-hydrate per 100 g. water. The difference between the amount of egg-hydrate thus estimated and that actually found is equal to 0.097 g. and may, at least partly, be attributable to differences in the conditions of crystallisation and, consequently, in the velocity of the latter. If, however, the whole difference be supposed to originate in the formation of non-crystallisable albumin, then a simple calculation will give the result that the amount of the latter only makes out about $^1/_2$ % of the whole amount of egg-albumin present in the original solution. We therefore feel justified in asserting that the amount of non-crystallisable albumin formed in the course of dialysis is quite insignificant.

For the sake of completeness it is to be added that non-coagulable proteins could not be shown to exist in the filtrate, and, further, that the original solution was quite inodorous after the toluol had been expelled by a current of hydrogen.

The matter was quite different in the case of another sample of dialysed egg-albumin solution, which after removal of the toluol had a distinct, although barely perceptible foul smell. A similar crystallisation experiment as that described above showed that this solution, after being left to stand for a fortnight at the ordinary temperature, gave a filtrate of a hydrogen-ion concentration corresponding with $p_H = 4.99$ and containing per

$$100 \text{ g. water} \begin{cases} 1.593 \text{ g. egg-hydrate} \\ 30.859 \text{ - ammonium sulphate.} \end{cases}$$

As such a filtrate was estimated to contain about 0.140 g. crystallisable egg-albumin, the amount of non-crystallisable albumin per 100 g. water must be about 1.453 g. From this it was easy to calculate that in this case about 27 % of the whole amount of protein in the original solution was transformed into non-crystallisable albumin.

Further, the filtrate contained *non-coagulable proteins* corresponding to 2.7 mg. nitrogen per 100 g. water, *i. e.* not great, but certainly distinguishable quantities.

In conclusion, it may be noted that the above sample of dialysed egg-albumin was also used, in the manner described in Paper II, for some estimations of its capacity to combine with acids, with the result that the capacity, as expected, was markedly beyond the normal (see also Paper II).

Another crystallisation experiment made with the same sample of dialysed egg-albumin, from which the toluol was removed by a current of hydrogen, gave much the same result as the first experiment.

C. The Method of Proportionality.

If an egg-albumin solution is precipitated by ammonium sulphate, if the precipitate crystallised out after some days is filtered off, and if weighed parts of the filtrate as well as of the precipitate with the adherent mother liquor are analysed, then it will be possible from the results of the analyses to draw certain conclusions concerning the composition of the precipitate, on the presumption that the mother liquor surrounding the precipitate has the same composition as the filtrate[1]). In fact, supposing the proportion between the weights of ammonium sulphate and water to be the same for the filtrate and for the crystals with adherent mother liquor, it may safely be inferred that the crystals contain neither water nor ammonium sulphate, or else that they contain both these substances, and in the same mutual proportion as that in which they are contained in the mother liquor. On the other hand, if the analyses show the said proportion to differ, for instance in such a way that the filtrate for a certain amount of ammonium sulphate contains less water than the precipitate, it means that the crystals contain water. Besides this excess of water, they may also contain ammonium sulphate and water in the mutual proportion in which these substances exist in the filtrate. In the present chapter we are to deal with the application of the principle outlined above, which we designate as the "Method of Proportionality", while basing our considerations, for the sake of clearness, provisionally upon the example just mentioned.

We may point out beforehand that an analysis like that above described at once shows that the egg-albumin crystallised out contains considerable amounts of water[2]); we do not for the

[1] For this assumption to be right, it is necessary, in the practical execution of such an experiment, to take certain precautions. More particularly, the filtration must be arranged in such a way that the evaporation of water is precluded, and the first portion of the filtrate which has run through is not to be used, as — on account of the adsorbing power of the filter paper — it may have a different composition from that of the main filtrate (see Paper III).

[2] As regards the amount of water considered, we may (while referring, as for details, to Paper III) confine ourselves here to the remark that, whilst pure dried egg-albumin or coagulated egg-albumin contains 15.51 % nitrogen (Thomas B. Osborne and George F. Campbell: Journ. Americ. Chem. Soc., **22**, 440 (1900)), so that the factor by which the protein nitrogen has to be multiplied in order to give the weight of the egg-albumin is 6.45,

moment consider the question as to whether the crystals contain also ammonium sulphate plus water in the proportion in which these exist in the filtrate.

Let the weight of

ammonia nitrogen	in	100	g.	filtrate	be	a_f
„	„	„	„	precipitate	„	a_b
protein nitrogen	„	„	„	filtrate	„	p_f
„	„	„	„	precipitate	„	p_b,

and let x be the factor by which the protein nitrogen has to be multiplied to give the weight of hydrous egg-albumin, "egg-hydrate".

100 g. filtrate will then consist of

$a_f \cdot 4.7163$	g. ammonium sulphate[1])
$p_f \cdot x$	g. egg-hydrate[2])
and $(100 \div a_f \cdot 4.7163 \div p_f \cdot x)$	g. water

100 g. precipitate (with adherent mother liquor) *will*, conformably, *consist of*

$a_b \cdot 4.7163$	g. ammonium sulphate
$p_b \cdot x$	g. egg-hydrate
and $(100 \div a_b \cdot 4.7163 \div p_b \cdot x)$	g. water.

According to the Principle of Proportionality we have

$$\frac{a_f \cdot 4.7163}{100 \div a_f \cdot 4.7163 \div p_f \cdot x} = \frac{a_b \cdot 4.7163}{100 \div a_b \cdot 4.7163 \div p_b \cdot x},$$

from which

$$x = \frac{100\ (a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f} \qquad (1).$$

The formula (1) is calculated, and therefore only applicable, on the assumption that the egg-hydrate crystallised contains only

we found on the other hand that the factor by which the protein nitrogen has to be multiplied to give the weight of the *crystallised hydrous egg-albumin* is 7.86; the amount of water per 1 g. egg-albumin being thus 0.22 g.

[1]) 4.7163 is the factor by which ammonia nitrogen has to be multiplied to give the weight of the corresponding amount of ammonium sulphate.

[2]) We presuppose that the slight amount of egg-albumin present in the mother liquor has the same composition as the crystals separated. This assumption is probably not quite correct; yet, as the amount of egg-hydrate contained in the mother liquor generally forms but a fractional part of that of the precipitate, the error, if error there be, will be of no significance.

water. but no ammonium sulphate. We have now to examine how matters stand when the egg-hydrate crystallised out contains, besides water, some ammonium sulphate and water in the mutual proportion in which these substances are present in the mother liquor.

If we use the same designations as above, x is the factor by which the protein nitrogen has to be multiplied to give the weight of the crystallised egg-hydrate minus the weight of the ammonium sulphate forming part of it; y is the factor by which the protein nitrogen has to be multiplied to give the weight of ammonium sulphate included in the egg-hydrate, and z is the factor by which the protein nitrogen has to be multiplied to give the total weight of the crystallised egg-hydrate. Then we have:

$$z = x + y \qquad (2),$$

and *100 g. filtrate will contain*

$(a_f \cdot 4.7163 \div p_f \cdot y)$	g. ammonium sulphate
$p_f \cdot z$	g. egg-hydrate (containing water and ammonium sulphate)
$(100 \div a_f \cdot 4.7163 + p_f \cdot y \div p_f \cdot z)$	g. water.

100 g. precipitate (with adherent mother liquor) *contain*

$(a_l \cdot 4.7163 \div p_b \cdot y)$	g. ammonium sulphate
$p_b \cdot z$	g. egg hydrate (containing water and ammonium sulphate)
$(100 \div a_b \cdot 4.7163 + p_b \cdot y \div p_b \cdot z)$	g. water.

According to the principle of proportionality we have

$$\frac{a_f \cdot 4.7163 \div p_f \cdot y}{100 \div a_f \cdot 4.7163 + p_f \cdot y \div p_f \cdot z} = \frac{a_b \cdot 4.7163 \div p_b \cdot y}{100 \div a_b \cdot 4.7163 + p_b \cdot y \div p_b \cdot z}.$$

This equation gives by a simple calculation

$$z = -\frac{100\,(a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f} + y \cdot \frac{100\,(p_b \div p_f)}{4.7163\,(a_f \cdot p_b \div a_b \cdot p_f)} \qquad (3).$$

Equation (3) may then be written

$$z = r + s \cdot y. \qquad (4),$$

where the values of the co-efficients r and s are given in Equation (3).

Equation (4) is indeterminate, being satisfied by infinitely many values of y, each of which gives a corresponding value of z; the value $y = 0$ makes the Equations (3) and (4) identical with Equation (1), so that in this case — as might be expected — we have $z = r = x$.

It will thus be impossible to find the two unknown quantities z and y from a single experiment, the analytical results of which only permit a calculation of r and s; in many cases it is impossible to advance farther in this way, and it will then be necessary to resort to considerations of quite a different nature, which we shall not enter into here. It is, however, often possible simply by the use of the method of proportionality to advance a step forward by determining an approximative value of y. As y is very small and the term $s \cdot y$ generally represents only a little correcting term, we may often be able to find a nearly correct value of z. This will, for instance, be the case when two experiments made in quite the same manner, only with a little difference in the ammonium sulphate concentrations used, are found to give different values as well for r (r_1 and r_2) as for s (s_1 and s_2). Supposing that z and y have the same value in one experiment as in the other, we have

$$z = r_1 + s_1 \cdot y = r_2 + s_2 \cdot y$$

$$y = \frac{r_1 \div r_2}{s_2 \div s_1} \qquad (5).$$

The value found for y is, of course, only an approximate one; but it will be nearly correct for an experiment with an ammonium sulphate concentration intermediate between those used in the two experiments mentioned. When y has been found, z can be calculated, and then x can be found also, since $z = x + y$.

We are now going to examine somewhat more closely the co-efficients, r and s, which enter into the Equations (3) and (4):

$$r = \frac{100\,(a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f}, \quad s = \frac{100\,(p_b \div p_f)}{4.7163\,(a_f \cdot p_b \div a_b \cdot p_f)}$$

As regards the denominators in these co-efficients, a_f will, under the particular conditions in question, always be greater than a_b, and as p_f is always very small as compared with p_b, it will very often be possible to discard the term $a_b \cdot p_f$. At any rate, the magnitude of the denominator is almost entirely deter-

mined through the term $a_f \cdot p_b$. The co-efficient r may then, in a reduced and practically correct form, be written thus:

$$r = \frac{100\,(a_f \div a_b)}{a_f \cdot p_b} = \frac{100}{p_b}\,(1 \div \frac{a_b}{a_f}).$$

From the formula, written in this way, it appears that an error made in the determination of p_b will act with all its weight, but not more, so that for instance an error of 1 % in the determination of p_b will give an error of 1 % in the value of r.

In regard to a_b and a_f, it is seen that the errors per cent relating to these two quantities neutralize each other when they are equal and go in the same direction. If, on the contrary, one of these quantities, but not the other, is subject to an error, this may act — it depends on the ratio $\frac{a_b}{a_f}$ — with several times its own weight. If this ratio is for instance about 0.8 — which may often be the case —, it is seen that an error of 1 % in the value of a_b (but not of a_f) will act four times more strongly on the difference $(1 \div \frac{a_b}{a_f})$, which will have a value of about 0.2, and accordingly the error in the value of r will amount to 4 %. Hence it is of great importance to execute the determinations of ammonia in the precipitate and in the filtrate at the same time and in exactly the same manner, in order to be sure that any sources of error have exerted the same influence in either case (see Section D p. 48).

As for the co-efficient s, it may be written

$$s = \frac{100 \cdot p_b}{4.7163\ a_f \cdot p_b} = \frac{100}{4.7163 \cdot a_f}$$

if the term $a_b \cdot p_f$ in the denominator and the comparatively small term p_f in the numerator are discarded.

It appears from this formula that the error in the value of s depends solely on the error in the determination of a_f.

As $4.7163 \cdot a_f$ is the weight of the amount of ammonium sulphate in 100 g. filtrate, s is the factor by which the weight of ammonium sulphate has to be multiplied to give the whole weight of the filtrate in which the ammonium sulphate exists. As, further, y is the factor by which the protein nitrogen has to be multiplied to give the weight of the ammonium sulphate in-

cluded in the egg-hydrate, it follows that $s \cdot y$ will be the factor by which the protein nitrogen has to be multiplied to give the weight of the filtrate included in the egg-hydrate corresponding to the protein nitrogen. Thus the right side of the equation

$$z = r + s \cdot y$$

consists, formally, of two terms, the first of which, r, represents the factor by which the protein nitrogen has to be multiplied to give the weight of the egg-albumin plus the "surplus" water in the egg-hydrate, whilst the term $s \cdot y$, as mentioned above, is the factor by which the protein nitrogen has to be multiplied to give the weight of the filtrate included in the egg-hydrate. In these considerations we have used the formula for s corresponding with $p_f = 0$; but it will be easily seen that even if p_f has a measurable value, a_f will always (if p_f is small as compared with p_b) have by far the greatest influence on the value of s. In such cases we may therefore make quite similar reflections as for the limit case $p_f = 0$.

We have hitherto assumed that the egg-hydrate has combined with the two constituents of the ammonium sulphate in equivalent quantities; but this will only take place in some special cases. The formulas set forth above may, however, also be used in such cases where ammonia and sulphuric acid are not bound in equivalent quantities, if only the meaning of the factors x and y is slightly modified.

We shall first examine the case where the equivalent concentration of the fixed sulphuric acid is greater than that of the fixed ammonia (as will occur at hydrogen-ion concentrations greater than $10—20 \cdot 10^{\div 6}$ (see Paper II)). The whole amount of fixed ammonia may here be considered as combined in the form of ammonium sulphate, exactly as assumed in the above calculations, while the "surplus" sulphuric acid enters into these as fixed water. In this case x will thus be the factor by which the protein nitrogen has to be multiplied to give the weight of hydrous egg-albumin plus surplus sulphuric acid. If now the fixed surplus sulphuric acid per gram-equivalent of protein nitrogen corresponds with q cc. $^n/_1$ sulphuric acid[1]), it means that 14.01 g. protein nitrogen are combined with $q \cdot 0.04904$ g. sulphuric acid.

[1]) Concerning the method of determination of the fixed surplus sulphuric acid or ammonia, the reader is referred to Paper II.

The factor by which the weight of the protein nitrogen has to be multiplied to give the weight of the fixed surplus sulphuric acid, will then be $q \cdot \frac{0.04904}{14.01} = 0.0035 \cdot q$; and the factor by which the weight of the protein nitrogen has to be multiplied to give the weight of the hydrous but ammonia- and sulphuric acid-free egg-albumin will be $x \div 0.0035 \cdot q$.

In cases where — at hydrogen-ion concentrations lower than $10—20 \cdot 10^{\div 6}$ — more ammonia than sulphuric acid is fixed, and — as assumed in the above formulas — the whole amount of fixed ammonia is calculated as combined in the form of ammonium sulphate, the value of y found by the calculation will be too high and that of x too low, since an amount of water of the same weight as the amount of sulphuric acid equivalent to the "surplus" ammonia is included in the weight of the ammonium sulphate combined with the protein. If now the amount of surplus ammonia combined with one gram-equivalent protein nitrogen is m cc. $^{n}/_{1}$ ammonia, the weight of sulphuric acid equivalent to this ammonia will be $m \cdot 0.04904$ g., and the factor by which the protein nitrogen has to be multiplied to give the weight of this quantity of sulphuric acid will be $m \cdot \frac{0.04904}{14.01} = 0.0035 \cdot m$. Both x and y must in this case be corrected in such a way that the factor by which the protein nitrogen has to be multiplied to give the weight of the *hydrous, but ammonia- and sulphuric acid-free* protein becomes $x + 0.0035 \cdot m$, while the factor by which the protein nitrogen has to be multiplied to give the weight of the fixed ammonium sulphate plus the surplus ammonia becomes $y \div 0.0035 \cdot m$. If, finally, the question is how this latter factor is to be divided when the amount of ammonium sulphate as well as that of surplus ammonia are to be calculated, then quite a similar calculation to that mentioned above will give the result that the weight of the amount of ammonia in m cc. $^{n}/_{1}$ ammonia is $m \cdot 0.017034$ g., and consequently the factor by which the protein nitrogen has to be multiplied to give the weight of fixed surplus ammonia will be $m \cdot \frac{0.017034}{14.01} = 0.0012 \cdot m$, so that the factor by which the protein nitrogen has to be multiplied to give the weight of fixed ammonium sulphate will be $y \div 0.0035 \cdot m \div 0012 \cdot m = y \div 0.0047 \cdot m$.

Thus it is possible in all cases to calculate x and y as stated

above, and z is always equal to $x + y$; but the significations of x and y are different in the different cases. Table 2 gives a general view of the matter.

TABLE 2.

The egg-albumin contains per gram-equivalent protein nitrogen	The factor by which the protein nitrogen has to be multiplied to give the weight of			
	hydrous but sulphuric acid- and ammonia-free egg-albumin	ammonium sulphate fixed	surplus sulphuric acid fixed	surplus ammonia fixed
neither surplus sulphuric acid nor ammonia	x	y	—	—
q cc. surplus $^{n}/_{1}$ sulphuric acid	$x \div 0.0035 \cdot q$	y	$0.0035 \cdot q$	—
m cc. surplus $^{n}/_{1}$ ammonia	$x + 0.0035 \cdot m$	$y \div 0.0047 \cdot m$	—	$0.0012 \cdot m$

In the preceding pages we have constantly been considering a special example, *viz.* the crystallisation of egg-hydrate by addition of ammonium sulphate; but the method of proportionality is applicable to a variety of other cases too.

Thus we have used the method — though for the present only in some few experiments — in investigations on the composition of heat-coagulated egg-albumin. If 100 g. of an ammonium sulphate containing egg-albumin solution before coagulation contains p_b g. protein nitrogen and a_b g. ammonia nitrogen, and if the coagulation is effected in such a manner that no water or ammonia has evaporated, then 100 g. of the coagulated mixture must, of course, also contain p_b g. protein nitrogen and a_b g. ammonia nitrogen. If further, the coagulated egg-albumin is filtered off, and if the analysis of the filtrate shows that 100 g. of the latter contain p_f g. protein nitrogen and a_f g. ammonia nitrogen, it is evidently possible on the basis of these analyses to deduce exactly the same formulas as those in the more explicit example given above.

No less interesting are the applications which can be made of the method of proportionality in the study of the colloidal solution itself. If, for instance, an egg-albumin solution containing

ammonium chloride, but otherwise pure, is treated with an excess of solid ammonium chloride, the egg-albumin will not be precipitated, but an equilibrium will be brought about between the two phases of the colloidal solution, and it will then be possible by the use of the method of proportionality to draw conclusions as to the composition of the disperse phase. In this case the "filtrate" is represented by the dispersion medium consisting of an ammonium chloride solution saturated at the experimental temperature, and consequently having a composition which can be easily ascertained. The disperse phase with surrounding dispersion medium, that is, the colloidal solution itself, corresponds to the "precipitate with adherent mother liquor", and the analysis of this solution can be made after the excess of ammonium chloride has been filtered off with proper precautions. In experiments of this kind — which we have not, however, as yet had occasion to make — ammonium sulphate cannot, of course, be used, as the egg-albumin is precipitated completely by saturation of the solution with ammonium sulphate.

The method, however, acquires a much larger range of application by the introduction of a semi-permeable membrane between the colloidal solution and the pure dispersion medium. With this modification we have used the method in a very large number of experiments, which will be described in Paper V, and in which the colloidal solution experimented upon (in most cases an egg-albumin solution containing ammonium sulphate) was placed in a collodion cap, which was suspended in an ammonium sulphate solution adapted to the composition of the egg-albumin solution. The experiment was made in such a manner that a pressure was exerted upon the egg-albumin solution, the "inner liquid", this pressure being equal to the osmotic pressure of the solution, and the cap was kept at a constant temperature for several days in the ammonium sulphate solution, the "outer liquid", in order to produce a complete distribution-equilibrium between the inner and the outer liquids. Thereafter these two liquids were analysed, and the results obtained were worked up on the basis of the method of proportionality; in this case the outer liquid corresponded with the "filtrate" and the inner liquid with the "precipitate with adherent mother liquor".

There can hardly be any doubt that the method in this form may be applicable in investigations on a variety of colloidal so-

lutions, and probably it will be possible in this way to obtain important information as to the nature of the disperse phase, and more especially concerning the character and range of such alterations as may be caused in the composition of the disperse phase by modifications occurring in the composition of the dispersion medium.

D. Methods of Determination of Nitrogen.

It will have been noticed in the foregoing chapters that the quantitative treatment of the questions discussed in the present papers is essentially to be based upon a series of determinations of the protein and ammonia nitrogen contained in the solutions or mixtures of crystals and mother liquor to be examined. The determination of the amount of nitrogen in non-coagulable proteins is of minor importance, this determination being generally only made for the purpose of proving that proteins of this kind are not present.

We have, of course, in every manner tried to attain the greatest possible *absolute* exactness in the execution of these determinations; but in not less degree we have made it the object of our endeavours to obtain the same *relative* precision in such analyses where this may be of some significance. In the previous section, when mentioning the determination of the factors x, y and z by means of the method of proportionality, it was emphasized that in investigations of this kind it is of the greatest importance that the errors made in the determinations of ammonia nitrogen in the "filtrate" and in the "precipitate with adherent mother-liquor" or in the "inner liquid" and in the "outer liquid" should be of the same order of size. In such analyses we have therefore endeavoured to obtain the same degree of exactness by a completely identical treatment of both the solutions experimented upon. Thus, to take a special example, in the analysis of an inner liquid containing both ammonium sulphate and *protein* and of the corresponding outer liquid containing ammonium sulphate *but no protein*, we have experimented on such quantities that the amount of ammonia nitrogen was practically the same in the samples of both solutions, and then we have treated all the samples in exactly the same manner and just as, after addition of acetic acid and sodium acetate (see p. 50),

we heat the inner liquid in order to coagulate the egg-albumin, so also we treat the outer liquid in the same way and heat it for the same length of time, even if no precipitate has been hereby thrown down. We filter all the samples at the same time on filters of the same kind and size, and wash the precipitates derived from the samples of the inner liquid at the same time and with the same quantity of water as the empty filters used for the samples of the outer liquid. Finally, in distilling off the ammonia, and in the subsequent titration, we alternately distill the samples of inner liquid and outer liquid. By so doing, eventual sources of error are, we believe, rendered as nearly as possible of the same consequence in the analyses of both solutions. This is, as before mentioned, of very considerable importance (compare also p. 60).

As the amount of egg-albumin corresponding with 20 mg. of protein nitrogen is suitable for a Kjeldahl determination, we have as far as possible chosen the weighed samples of inner liquid or of solutions of precipitate for the analyses in such a way that the amount of protein nitrogen was 15—20 mg. The amount of ammonia in the filtrate derived from the coagulated egg-albumin accordingly sometimes turned out to be very great, sometimes quite minimal, and the determination of ammonia nitrogen was therefore made in different ways. If the amount of ammonia present was very small, the ammonia nitrogen was determined in a larger portion, which contained too much egg-albumin for a Kjeldahl determination; besides, the amount of total nitrogen was determined by Kjeldahl's method in smaller samples, and the amount of protein nitrogen was then equal to the difference between total nitrogen and ammonia nitrogen.

In the following pages we give an account of the details of the methods used in the different cases, and finally we shall describe a series of control experiments effected with a view to elucidating the exactness of our analytical methods.

a. Determination of the amount of Total Nitrogen

In a long-necked flask (250 cc.) made of *Jena*-glass a measured or weighed quantity of the solution to be analysed is heated over a well-heating *Argand* burner, together with 20 cc. of concentrated sulphuric acid, 5 g. potassium sulphate and a

small bit of copper wire (about 50 mg.). To begin with, we heat but slightly till the water has evaporated; later on, the temperature is raised so as to maintain the liquid at or very near its boiling point. While repeatedly shaking — in order to bring down all the carbonised particles into the liquid, that they may be completely decomposed —, the liquid is heated till it has acquired a marked green colour (2 to 4 hours), when the heating is continued for 5 hours more. Then the still boiling liquid is oxidised by adding cautiously three times about 0.5 g. of dry, pulverised potassium permanganate, the flask being well shaken after each addition. After being completely cooled, the liquid is diluted with water, and the ammonia distilled off as usual.

As "blank tests", experiments are made with the same quantities of the reagents employed, which receive an admixture of 5 drops of a solution of pure cane sugar (10 %). The "blank tests" are boiled at the same time as the real analyses, and are also oxidised and distilled in quite the same manner.

b. Determination of the amount of Nitrogen in the Coagulable Proteins present.

In a small conical flask (about 150 cc.) a measured or weighed quantity of the solution in hand for analysis is mixed with 10 cc. $^n/_1$ acetic acid, 10 cc. $^n/_1$ sodium acetate solution[1]) and water up to a total volume of about 100 cc., after which the mixture, while being repeatedly shaken, is coagulated by heating on a boiling water-bath for $^1/_2$ hour. After cooling[2]), the precipitate is filtered off on a filter (as far as possible free from nitrogen and washed with hot water; we use the mark "C. Schleicher & Schüll Nr. 589" — 12.5 cm.) and washed carefully with hot water. Then the filter with the precipitate is put into the decomposition

[1]) If the filtrate is to be used for determination of the ammonia nitrogen, the ammonia is prevented from evaporating during the coagulation by adding before coagulation *only* 10 cc. $^n/_1$ acetic acid, *2 cc. $^n/_1$ sodium acetate solution* and water, whilst the remaining *8 cc. $^n/_1$ sodium acetate solution should not be added till the day before the filtration,* for the sake of obviating infection. At the hydrogen-ion concentration contingent upon the "buffer"-mixture 10 cc. $^n/_1$ acetic acid + 10 cc. $^n/_1$ sodium acetate the coagulated egg-albumin is completely precipitated.

[2]) Provided that, after coagulation, the flask is covered with a glass plate, without being cooled, it may safely be left to stand for many days before filtration.

flask and treated as described under *a*, the sulphuric acid intended to effect the decomposition being first used for washing the flask and the funnel employed in the coagulation and filtration.

Similar experiments as stated under *a* are made as "blank tests", only with this difference that we do not add cane sugar, but a filter of the same kind, washed and treated in the same way as in the real analyses.

c. Determination of the amount of Nitrogen in the Non-coagulable Proteins present.

The nitrogenous substances contained in the filtrate[1]) of the coagulated protein consist in ammonium salts, associated with non-coagulable nitrogenous compounds non-volatile in alkaline liquid. The last-mentioned substances are comprised under the designation "non-coagulable proteins", and their amount is estimated by distilling off from the filtrate the ammonia in an alkaline liquid and then making a nitrogen determination by Kjeldahl's method in the remainder. The whole of the filtrate from the coagulated protein, or a measured portion, is mixed, in a spacious flask, with a little phenolphtalein and such a proportion of pure 2n sodium hydroxide solution that all the ammonia present is set free, and then the ammonia is distilled off *in vacuo* at a temperature of 30°. After evaporation nearly to dryness, the rest is again dissolved in water, and the solution must now show a markedly alkaline reaction; if this is not the case, a further addition of sodium hydroxide solution is necessary. All the parts of the evaporating apparatus are well washed, and the solution is then again evaporated nearly to dryness in the same manner as before; finally, this process is repeated a third, and in the case of filtrates rich in ammonium sulphate a fourth time. The completely ammonia-free remainder obtained in this way is by the aid of as little water as possible brought into one of the usual decomposition flasks, where concentrated sulphuric acid is added cautiously until the solution shows an acid reaction; then a further addition of 10 cc. has to ensue. The

[1] The filtrate is usually only able to keep free from mould for a few days, and as in the case of long series of experiments it is often necessary to be able to keep filtrates for analyses later on, we have always added *20 cc. 2n sulphuric acid to every filtrate* immediately after the filtration; *then the filtrates may be kept unaltered for a long time.*

water present is evaporated, and the nitrogen determination is then made as described under *a*, yet without oxidation with potassium permanganate.

As "blank tests", experiments are made in which pure water or an ammonium sulphate solution of suitable strength is evaporated as described above, with the same quantities of the same sodium hydroxide solution which was used in the analysis. In the remainder, a nitrogen determination is made at the same time and in the same manner as the analyses.

d. Determination of Ammonia Nitrogen if the amount is equal or superior to 50 mg.

The filtrate (abt. 500 cc.) obtained by the filtration and washing of the egg-albumin and to which, as above mentioned (see p. 51, note). 20 cc. 2n sulphuric acid is added, is poured into a weighed 1 l. flask and made up to the mark by addition of water, when the whole is weighed again. From this solution at least three equal samples are weighed off, each containing about 20 mg. of ammonia nitrogen. From these samples the ammonia is distilled off by means of sodium hydroxide and with abt. n/7 hydrochloric acid in the receiver, whereupon the titration is made iodometrically with abt. n/14.01 sodium thiosulphate in the usual manner[1]).

Simultaneously with the distillation of the samples, at least three weighed samples of a solution of pure ammonium sulphate dried at 70° *in vacuo* are distilled in order to determine the strength of the thiosulphate; the ammonium sulphate used may thus be considered as the *primary standard substance* for all the analyses. These samples are also chosen in such a way that each contains about 20 mg. ammonia nitrogen, and to each are added the same amounts of acetic acid, sodium acetate and sulphuric acid as contained in the samples to be analysed.

As "blank test" as well for the "analysis samples" as for the "standard samples", we distilled pure water to which the same amounts of acetic acid, sodium acetate and sulphuric acid as existing in the samples and sodium hydroxide had been added.

[1]) As for the principles underlying the method of iodometric titration of acids, the reader is referred to K. Koefoed: Comptes-rendus du Laboratoire de Carlsberg, **10**, 52 (1911).

The quantity of water to be distilled is chosen in such a way that the whole volume amounts to 300—325 cc., and to this is added 25 cc. concentrated sodium hydroxide. The distillate is received in 15 cc. abt. $^n/_7$ hydrochloric acid, and so great a volume is distilled off (150—180 cc.) that the distillate after the titration amounts to abt. 200 cc.

With the view of further eliminating any sources of error, we have always made the distillations in the following succession: the distillating apparatus is first cleaned by *evaporation*: distilling of pure water and sodium hydroxide, without letting on cooling water. Then the "blank tests" are distilled. The next distillation is made with an ammonium sulphate solution, which in the amount used contains ammonia in quantity practically equal to that of the samples experimented upon: it is not till then that the distillation of "analysis"- and "standard-samples" is proceeded with. This "preparatory" distillation, in which the receiver contains, as usual, 15 cc. $^n/_7$ hydrochloric acid, is intended to bring the distillating apparatus into exactly the same condition *before* the first distillation of "analysis"- or "standard-samples" as that in which it is *after* the first, consequently before the second distillation. The fact is that, even if the volume of the liquid to be distilled is kept within the limits mentioned, and even if the distillation is continued as far as above stated, it has been invariably found that quite minimal amounts of ammonia — which, however trifling, can still be detected by a Nessler test — will always be left partly in the distillation flask and partly in the washing and cooling apparatus. From this reason it is necessary to make the preparatory distillation above mentioned, if we are to be sure that all samples — "analysis" as well as 'standard samples" — are distilled under quite the same conditions, so that eventual errors influence the standardisation of the thiosulphate and the analyses themselves in the same degree and therefore neutralise their reciprocal effects.

e. Determination of Ammonia Nitrogen in quantity from 25 to 50 mg.

Filtrate and washing water are poured into a graduated flask, made up to 500 cc. and well shaken; then the liquid is by means of a 250 cc. graduated flask divided into two nearly equal parts. Each of these is distilled and titrated separately after each flask

has been washed with 50—75 cc. water. *The amount of ammonia nitrogen in the filtrate will thus be equal to the sum of those found by the two titrations.* As for the rest, the procedure is the same as described under *d*, only with this difference that the amount of ammonia contained in the "standard samples" should, of course, be much the same as the amount of ammonia in one half of the filtrate.

f. Determination of Ammonia Nitrogen when the amount is 10 to 25 mg.

In this case the volume of the filtrate after addition of the 20 cc. 2n sulphuric acid should be abt. 300 cc., and the whole of the filtrate is distilled at one time. The amount of ammonia in the "standard samples" should be about the same as in the total filtrate; as for the rest, the proceeding is exactly the same as above described.

g. Determination of the Ammonia Nitrogen when the amount is less than 10 mg.

The less the amount of ammonia in the filtrate, the greater will be the effect of the source of error above mentioned: that after distillation, minimal amounts of ammonia will remain both in the distilling flask and in the washing and cooling apparatus, and we cannot, of course, reckon upon these small amounts being absolutely equal, even if the amounts of ammonia to be distilled are equal, and even if the distillation is carried out always, as far as possible, in the same manner. Moréover, in the case of egg-albumin solutions very poor in ammonium sulphate, it is necessary to analyse an abundant portion of the solution[1]) in order to have a fairly sufficient amount of ammonia for the determination. The coagulation must then, on account of the great amount of egg-albumin, be effected after addition of a somewhat greater volume of water than usual (150—200 cc.), and for the same reason the precipitate must be washed with larger quantities of water. The volume of the filtrate will consequently be such that we shall have some difficulty in distilling off the am-

[1]) In such cases the protein nitrogen is estimated as the difference between the total nitrogen (determined in especial samples by the method described under *a*) and the ammonia nitrogen.

monia so completely by a single distillation as to justify us in leaving out of consideration the above-mentioned source of error. In this case we therefore use a somewhat more complicated method.

The coagulated egg-albumin is filtered off and washed as usual; but when the aggregate volume of the filtrate and washing water has reached abt 500 cc. (Filtrate I), the precipitate — if considerably greater than usual — is taken from the filter and well mixed up with hot water in a dish; then it is brought back to the filter and washed with hot water, until this second filtrate (Filtrate II) has a volume of 300 cc. The whole of Filtrate I is distilled all at once (the flask is washed with 25—30 cc. water), the ammonia is received in abt. $^{n}/_{35}$ hydrochloric acid, and the excess of hydrochloric acid is titrated iodometrically with abt. $^{n}/_{70.05}$ thiosulphate solution. The distillation is otherwise carried out as described *sub d*; but when this first distillation has been brought to an end, that is, when nearly all the ammonia has distilled, a long-necked 200 cc. graduated flask containing 5 cc. $^{n}/_{7}$ hydrochloric acid is placed as receiver, and then the distillation is continued till the distillate fills the graduated flask up to the mark. The amount of ammonia in this second distillate is determined by Nessler's method by addition of 5 cc. $^{n}/_{5}$ sodium hydroxide solution and 2 cc. alkaline solution of potassic mercuric iodide[1]). The distillate obtained by the distillation of Filtrate II is collected in the same way in a 200 cc. graduated flask, and the amount of ammonia is determined according to Nessler.

In the cases just-mentioned, the distillation is always carried out by the aid of recently boiled sodium hydroxide, and it need not be added that we try by every means to treat the "analysis samples" and the "standard samples" in exactly the same way. As *liquids for comparison* in the colorimetric Nessler *determination* we use samples containing known quantities of ammonium sulphate. Each sample receives an addition of 5 cc. $^{n}/_{7}$ hydrochloric acid and is made up with water to 200 cc., when 5 cc. $^{n}/_{5}$ sodium hydroxide solution and 2 cc. potassic mercuric iodide solution are added. The strength of colour of the "Nessler ana-

[1] Concerning the preparation of the solution of potassic mercuric iodide the reader is referred to A. Classen: Analytische Chemie II, 115 (1903).

lyses", as compared with the strength of colour of the liquids for comparison, is determined by means of a common colorimeter the day after preparation of the samples.

h. Control experiments concerning the Determination of Ammonia Nitrogen.

These control experiments had for their object to enable us to judge of the degree of exactness obtainable by the different methods used for the determination of ammonia, and also to ascertain whether the determination of ammonia can be effected with the same exactness *in the presence as in the absence of egg-albumin.*

As *stock-solution* was used an ammonium sulphate solution which a previous careful analysis had shown to contain:

783.6 mg. ammonia nitrogen per 100 g. solution.

The egg-albumin solution employed was prepared from crystallised egg-albumin purified as usual by six crystallisations with ammonium sulphate, and then freed from ammonia by six crystallisations with potassium and sodium sulphate[1]). 100 cc. of the albumin solution contained 226.6 mg. protein nitrogen, but only 0.03 mg. ammonia nitrogen.

Three series of experiments were made with different concentrations of ammonium sulphate, and, consequently, different methods were used for the determination of ammonia.

Series of experiments A.
(Experiments Nos 1 to 6).

For each experiment a weighed quantity — 50 cc. — of the stock-solution was used.

In the experiments Nos. 1 to 3 no egg-albumin, but only acetic acid, sodium acetate and sulphuric acid were added to the solution, and the determination of ammonia was then made by *d.*

In the next three experiments (Nos. 4 to 6) 10 cc. egg-albumin solution, and further acetic acid and sodium acetate (see p. 50, note 1), were added to the solution, which was then coagu-

[1]) The crystallisation of the egg-albumin by means of other substances than ammonium sulphate will be dealt with in a following paper.

lated by *b*. After filtration, the ammonia nitrogen was determined by *d*.

The results are set forth in Table 3.

TABLE 3.

Series of Experiments A.

(Concentration of the Thiosulphate solution: 1.0022 · $n/_{14.01}$).

Number of Experiment	Weight of 50 cc. of the stock solution	Weight after dilution to 1 l.	Weight of the 3 · 50 cc. used for distillation	On titration an amount of ammonia was found correspond. with *a* cc. of the thiosulphate solution
	g.	g.	g.	*a*
Without egg-albumin				
1.....	50.9466	1000.01	49.8106 49.8206 49.7794	19.90 19.88 19.86
2.....	50.9504	1000.01	49.8085 49.8057 49.7971	19.85 19.85 19.90
3.....	50.9480	1000.23	49.8315 49.8058 49.8677	19.91 19.85 19.92
Average...	50.9483	1000.08	49.8141	19.880
100 g. of the stock solution thus contain 785.1 mg.[1] ammonia nitrogen.				
With egg-albumin (22.66 mg. protein nitrogen)				
4.....	50.9592	1000.04	49.7904 49.7854 49.7914	19.91 19.91 19.87
5.....	50.9678	1000.21	49.8000 49.7977 49.7758	19.88 19.87 19.84
6.....	50.9512	1000.21	49.7976 49.8522 49.8206	19.90 19.89 19.89
Average...	50.9594	1000.15	49.8012	19.884[2]
100 g. of the original solution thus contain 785.3 mg. ammonia nitrogen.				

[1] $\frac{19.880 \cdot 1.0022 \cdot 1000.08 \cdot 100}{49.8141 \cdot 50.9483} = 785.1.$

[2] Here the correction for the amount of ammonia in the egg-albumin is minimal, namely 0.003 · $50/_{1000}$ mg. ammonia nitrogen.

Series of experiments B.

(Experiments Nos. 7 to 14).

50 cc. of the stock-solution was weighed off (weight: 50.9310 g.) and diluted to 1 l. in a standardised graduated flask. For each experiment 50 cc. was taken, without weighing, from the diluted solution with a standardised pipette.

In the experiments Nos. 7 to 10 egg-albumin was not added, and the determination of ammonia was made after *f*.

In the experiments Nos. 11 to 14 10 cc. egg-albumin solution was added. After coagulation etc. the ammonia nitrogen was determined after *f*.

The results are set forth in Table 4.

TABLE 4.

Series of Experiments B.

(Concentration of the Thiosulphate solution: $1.0022 \cdot {}^{n}/_{14.01}$)

Number of Experiment	On titration an amount of ammonia was found corresponding with *a* cc. of the thiosulphate solution *a*	Number of Experiment	On titration an amount of ammonia was found corresponding with *a* cc. of the thiosulphate solution *a*
Without egg-albumin		With egg-albumin (22.66 mg. protein nitrogen)	
7.....	19.94	11.....	19.94
8.....	19.94	12.....	19.95
9.....	19.91	13.....	19.93
10.....	19.91	14.....	19.94
Average.....	19.925	Average.....	19.940[1])
100 g. of the stock solution thus contain 784.2 mg. ammonia nitrogen.		100 g. of the stock solution thus contain 784.6 mg. ammonia nitrogen.	

Series of experiments C.

(Experiments Nos. 15 to 26).

20 cc. of the stock-solution was weighed off (weight: 20.3881 g.) and diluted in a standardised graduated flask to 1 l. For each experiment 50 cc. — not weighed — was taken from the diluted solution with a standardised pipette.

[1]) Here the correction for the amount of ammonia in the egg-albumin solution is 0.003 mg. ammonia nitrogen.

In the experiments Nos. 15 to 18 egg-albumin was not added, and the determination of ammonia was made after *g*.

In the experiments Nos. 19 to 22 was added 10 cc. egg-albumin solution, and in the experiments Nos. 23 to 26 50 cc. After coagulation etc. the ammonia nitrogen was determined after *g*.

The results are set forth in Table 5.

TABLE 5.

Series of Experiments C.

(Concentration of the Thiosulphate solution: 1.0340 · $^n/_{70.05}$)

Number of Experiment	On titration an amount of ammonia was found corresponding with *a* cc. of the thiosulphate solution	The Nessler-determination gave an amount of ammonia corresponding to		Corrected amount of abt. $^n/_{70.05}$ thiosulphate solution
		n mg. nitrogen	*b* cc. abt. $^n/_{70.05}$ thiosulphate solution	
	a	*n*	$b = n \cdot \frac{5}{1.0340}$	$a + b$
Without egg-albumin				
15	38.80	0.023	0.111	38.911
16	38.65	0.033	0.160	38.810
17	38.55	0.065	0.314	38.864
18	38.70	0.061	0.295	38.995
			Average...	38.895
100 g. of the stock solution thus contain 789.0 mg. ammonia nitrogen.				
With some egg-albumin (22.66 mg. protein nitrogen)				
19	38.75	0.046	0.222	38.972
20	38.75	0.060	0.290	39.040
21	38.75	0.043	0.208	38.958
22	38.75	0.052	0.251	39.001
			Average...	38.993[1]
100 g. of the stock solution thus contain 790.7 mg. ammonia nitrogen				
With much egg-albumin (113.3 mg. protein nitrogen)				
23	39.00	0.043	0.208	39.208
24	38.85	0.063	0.305	39.155
25	38.95	0.032	0.155	39.105
26	38.75	0.069	0.334	39.084
			Average...	39.138[2]
100 g. of the stock solution thus contain 792.5 mg. ammonia nitrogen.				

[1] Here the correction for the amount of ammonia in the egg-albumin solution is 0.003 mg. ammonia nitrogen ∞ 0.015 cc. of the abt. $^n/_{70.05}$ thiosulphate solution.

[2] The correction is 0.015 mg. ammonia nitrogen ∞ 0.073 thiosulphate solution.

Thus 100 g. of the stock-solution contain:

		Ammonia nitrogen
After the original determination		783.6 mg.
Series of experiments A	without egg-albumin	785.1 „
	with „	785.3 „
Series of experiments B	without „	784.2 „
	with „	784.6 „
Series of experiments C	without „	789.0 „
	with some „	790.7 „
	with much „	792.5 „

From this table it appears that the determinations within each series of experiments exhibit a good mutual accordance, which is better than that existing between the different series of experiments. It has in the preceding pages been repeatedly emphasized that precisely the mutual accordance between the results of analyses of protein-free and protein-containing solutions is of paramount significance for the investigations in which we are here engaged. Even though the discrepancies observed within each series of experiments are hardly greater than the experimental error, a closer inspection of the tables will show that the determination has a marked tendency to become higher, the greater the amount of egg-albumin present during coagulation in proportion to the amount of ammonia. Whether this fact is due to experimental errors, or whether it has something to do with the coagulation of the egg albumin, cannot as yet be ascertained, but the question will be discussed in a following paper, dealing with the problem of coagulation generally.

As regards the accordance between the single series of experiments, it will be noticed that A and B agree well with the original determination, the greatest difference from the average (784.6 mg.) being here only abt. 1 : 800. The experimental error is greater when the amount of ammonium sulphate used for the determination is small, as in the series of experiments C (abt. 8 mg. nitrogen). As for the protein-free solution, the difference from the average above stated is about $^1/_2$ %, and as for the protein-rich solution even 1 %[1]). Therefore, if the amount of

[1]) The greater value of the nitrogen-content of the stock-solution found in the series of experiments C is not, of course, attributable to the circumstance that in this series we have made Nessler determinations, which increase the amount of ammonia found; for in standardising the $^n/_{70.05}$ sodium thio-

ammonia to be analysed is only small, it is hardly possible to reckon on a greater *absolute* exactness than that obtained above; yet also in such cases the error will probably go in the same direction in determinations executed at the same time and in the same way in protein-free and protein-containing solutions, and thus the error per cent in the difference of two such determinations will be of fairly the same size as in each determination.

i. Control Experiments concerning the Determination of Protein Nitrogen.

In these experiments it was our intention to elucidate the influence exerted by the variation of a series of details in the method used.

In all the experiments we used 10 cc. (measured with an exactness of 0.01 cc.) of an egg-albumin solution prepared by careful dialysis, and subsequent dilution with water, of a preparation which had been re-crystallised six times. 10 cc. of this solution contained 23—24 mg. protein nitrogen and 0.64 mg. ammonia nitrogen. In the greater part of the experiments the whole amount of nitrogen was determined; but in some experiments the protein nitrogen alone was determined, the egg-albumin being coagulated, filtered off and washed, and determination of nitrogen then made in the filter with the precipitate; to the amount of protein nitrogen found 0.64 mg. was added, so that the figures for these experiments also, as set forth in Table 6 (p. 64), give the whole amount of nitrogen.

All the series of experiments are set forth in Table 6 (p. 64). The designation *Kj.* in the second column means that the heating was carried out in the main after Kjeldahl's original method, the 10 cc. egg-albumin solution being heated in a long-necked *Jena*-flask (250 cc.) together with 20 cc. concentrated sulphuric acid and a small piece of copper wire (abt. 50 mg.). Whether a slight dose of potassium sulphate has likewise been added or not, is stated in the third column of the table, while the fourth column indicates how long a time the analysis was boiled beyond the time (2—4 hours) which had elapsed before the liquid had grown

sulphate solution we have made Nessler determinations in the same way, whereby the strength of the thiosulphate solution is calculated as correspondingly smaller.

green, a proof that the decomposition is practically accomplished. In the fifth column it is stated if after the boiling an oxidation with potassium permanganate was effected or not.

In the experimental series IX, X, XI and XII the egg-albumin was coagulated in the way described on p. 50, and the decomposition by sulphuric acid was only undertaken after filtration and washing. In two of these series (X and XII) 10 cc. saturated ammonium sulphate solution was added before the coagulation, in order to ascertain if the washing of the coagulated egg-albumin is satisfactory even in presence of so abundant an amount of ammonium sulphate.

The designation *Kj.-G.* means that the decomposition was brought about according to Gunning's modification: the 10 cc. egg-albumin solution was heated $^1/_2$ hour together with 20 cc. concentrated sulphuric acid, a small piece of copper wire and 5 g. potassium sulphate; then 15 g. potassium sulphate was further added, and the heating was continued for $2^1/_2$ hours more.

Kj.-G.-A. means that the decomposition was made after Gunning-Arnold's modification, that is in quite the same way as by the method of Gunning, only with the difference that $^1/_2$ g. mercuric oxide was added. In the series of experiments XV the ammonia was distilled off by means of a mixture of sodium hydroxide and sodium sulphide, a little cupric sulphate was added to the distillate in order to fix the trace of hydric sulphide present, and then the distillate was distilled once more in the ordinary way. In the series of experiments XVI only a single distillation with sodium hydroxide was carried out with the addition of a little grape-sugar (glucose), by which the present mercuric compounds are reduced into metallic mercury; for this very convenient proceeding we are indebted to Professor Dr. Ivar Bang, Lund.

It appears from the table, that *the oxidation with potassium permanganate cannot be omitted in the method of* Kjeldahl *in its simplest form* (the series of experiments I, II, III and IV); it is only in the experimental series IV — where 5 g. potassium sulphate was added and the boiling continued for 8 hours after the moment when the liquid grew green — that the correct result has been obtained, although one of the estimates is rather low. All the other experiments (series V-XIII), in which we used the simple method of Kjeldahl combined with an *oxidation* by

permanganate, may be said to have given satisfactory results. The average of the mean values of the found amount of nitrogen is for these series 24.40 mg., and none of the mean values differs by $^1/_2$ % from this average. In two series (IX and X) there is a single determination which is rather low; perhaps because 2 hours' boiling after the liquid has grown green is not always sufficient; or it may be due to experimental errors[1]).

From the series IX, X, XI and XII it is evident, that the *whole amount of egg-albumin is precipitated by the coagulation*[2]) and can be determined as exactly in this way as by direct decomposition. Further, the result proves to be uninfluenced by the presence of abundant amounts of ammonium sulphate.

As for the last three series of experiments, we have not thought fit to extend the time of boiling beyond three hours, taking into account the investigations of R. Koefoed[3]), which showed that the loss of nitrogen is rather considerable if ample quantities of potassium sulphate are present during the boiling. The series of experiments XIV shows that a treatment by Gunning's method for three hours without subsequent oxidation with potassium permanganate is not sufficient to give a complete decomposition, since all the values found are more or less low. By the Gunning-Arnold method the decomposition is, on the contrary, — as was to be expected — easily completed in the course of three hours; the values of the contents of nitrogen agree with, and thus support, those found in the experiments V-XIII.

On the basis of these experiments, which perfectly confirm the experience gained in our laboratory in the course of time, we have adopted the method for determination of protein nitrogen described above *sub a* and *b* (p. 49 and p. 50). The reason why we have preferred this method to the somewhat speedier method of Gunning-Arnold is that a little nitrogen — as shown by

[1] In numerous analyses made in the course of several years it has occurred now and then that, in spite of the treatment being throughout as far as possible the same, a few determinations turned out too low. It has therefore been of some importance that in each analysis we had generally made at least three determinations, so that a single too low value could be rejected.

[2]) In two experiments it was determined (in the way described p. 51 under *c*) if it was possible to detect any non-coagulable protein in the filtrate derived from the coagulated egg-albumin; but the filtrate, after distilling off the ammonia, proved to be completely free from nitrogen.

[3]) Comptes-rendus du Laboratoire de Carlsberg, **10**, 63 (1911).

Determination of Nitrogen in egg-albumin by Kjeldahl's method, varied in different ways.

Series of Experiments	Designation of the method used and Remarks about the execution	Variations in details of the method			Number of Experiments	Amount of Nitrogen found milligrammes	
		Amount of K_2SO_4 used g.	Duration of boiling	Oxidation with $KMnO_4$		Minimal and maximal value	Average
I	*Kj.*....	0	1 hour aft. turning green	No oxidation	4	23.55 23.92	23.76
II	*Kj.*....	0	8 — — — —	—	4	23.72 24.05	23.90
III	*Kj.*....	5	1 — — — —	—	4	23.82 24.02	23.94
IV	*Kj.*....	5	8 — — — —	—	4	24.12 24.52	24.35
V	*Kj.*....	0	1 — — — —	Oxidation	4	24.32 24.43	24.37
VI	*Kj.*....	0	8 — — — —	—	4	24.36 24.48	24.42
VII	*Kj.*....	5	1 — — — —	—	4	24.43 24.46	24.45
VIII	*Kj.*....	5	8 — — — —	—	4	24.43 24.53	24.49
IX	*Kj.* Coagulation etc.	5	2 — — — —	—	6	24.10 24.48	24.32
X	*Kj.* Addition of 10 cc. conc. solution of ammonium sulphate, then coagulation etc.	5	2 — — — —	—	6	24.20 24.47	24.39
XI	*Kj.* Coagulation etc.	5	8 — — — —	—	6	24.34 24.47	24.42
XII	*Kj.* Addition of 10 cc. conc. solution of ammonium sulphate, then coagulation etc.	5	8 — — — —	—	6	24.30 24.47	24.41
XIII	*Kj.*....	5	5 — — — —	—	6	24.27 24.39	24.34
XIV	*Kj-G.*....	20	3 hours in all	No oxidation	5	23.46 24.22	23.84
XV	*Kj.-G.-A.* Double distillation, the first time with addition of sodium sulphide	20	3 hours in all	—	4	24.44 24.48	24.47
XVI	*Kj.-G.-A.* Single distillation with addition of glucose	20	3 hours in all	—	4	24.32 24.42	24.37

R. Koefoed[1]) — more easily disappears in the latter method than in that used by us, because the temperature of the boiling liquid is somewhat higher on account of the greater content of potassium sulphate. In fact the experiments of R. Koefoed show that the determination of nitrogen after the method of Kjeldahl must always be too low, since even pure ammonium sulphate, if heated together with concentrated sulphuric acid, loses nitrogen — unknown in what form —, and the more the higher the temperature is and the longer the time of heating. In a usual Kjeldahl determination, a six hours' heating caused a loss of about 1/8 % of the amount of nitrogen, whilst 24 hours' heating produced double the loss. In the method used by us we therefore suppose we shall have to reckon with a loss of about 1/2 % of the amount of nitrogen.

A great many of the experiments described in the following papers represent comparative investigations in which the absolute value of the concentration of protein is of secondary significance, and the error committed in the determination of nitrogen, which is always of the same magnitude when the same method is used, is therefore of no consequence worth mentioning. — If, on the other hand, some constant is to be determined, we must take into consideration the error inherent in the method. Of course, we must make the calculation with the values found for protein nitrogen; but it must always be borne in mind that these values are probably too low by about 1/2 %.

SUMMARY.

Section A.

1) The method used for the purification of egg-albumin from ash, mucoid and conalbumin is described in all detail, and it is explained how it is possible to follow quantitatively the progressive purification of the egg-albumin through repeated crystallisations with corresponding washings.

[1]) *loc. cit.*

2) It is shown that *the purification of egg-albumin from ash, mucoid and conalbumin does not offer any difficulties.* since none of these impurities are present in the egg-albumin in appreciable quantities after three crystallisations and washings. The whole of our initial material for the experiments described in the following papers is obtained from egg-albumin which has been crystallised at least six times, and therefore we feel justified in considering it as being free from the impurities just mentioned.

3) The purest commercial ammonium sulphate generally contains ash easily shown. Such preparations are not fit for use in the complete purification of the egg-albumin, especially in the last crystallisations and washings; for this purpose it is necessary to use solutions of ammonium sulphate preparations which are purified with special care and, if possible, completely free from ash.

4) Even the purest egg-albumin, if heated in a platinum dish, will leave a trace of ash; this does not, however, originate in any impurities, but in the phosphorus contained in the egg-albumin.

Section B.

1) A *dialysing apparatus* consisting of six dialysing cells is described in detail. The essential part of the dialysing cell is a *collodion cap* which is permeable to water and ammonium sulphate, but impermeable to egg-albumin. The apparatus is constructed in such a way that it is possible during the dialysis of the egg-albumin solution to establish a *diminished pressure* on "the outer liquid" (the water) in order to compensate the osmotic pressure of "the inner liquid" (the egg-albumin solution) and thus to prevent a dilution of the latter owing to absorption of water.

2) By means of this apparatus it is possible to free egg-albumin solutions containing ammonium sulphate from every trace of sulphuric acid and from the main part of the ammonia; the egg-albumin solution obtained by the dialysis may, after being exactly analysed, be used for the *preparation of egg-albumin solutions of well-defined composition.*

3) If *an egg-albumin solution* is guarded against the attack of micro-organisms by the admixture of an abundant quantity of toluol, and then shaken frequently and kept in ice in an ice-safe, *the solution will keep the characteristic properties of this particular protein unaltered for months.*

Section C.

If an egg-albumin solution is precipitated by ammonium sulphate and the precipitate crystallised out separated by filtration, it is possible, on the basis of analyses both of the filtrate and of the crystals with their adherent mother liquor, to draw conclusions concerning the composition of the crystals. The applicability of this method, which we have called the "*Method of Proportionality*", is thoroughly described with regard to the example here mentioned, it being also shown that the same principle may be applied to several other cases, more especially in connection with investigations on the nature of colloidal solutions. Suppose a colloidal solution to be placed in such a way that by means of a semi-permeable membrane it is separated from the pure dispersion medium, and suppose it further to be both in diffusion equilibrium and in osmotic equilibrium with the said medium; then the system may be treated in quite the same manner as above mentioned, the pure dispersion medium corresponding with the filtrate, and the colloidal solution corresponding with the crystals with adherent mother liquor.

Section D.

A thorough description is given of the *methods of nitrogen determination* used in this and the following investigations, and a series of *control experiments* are described, which were made in order to illustrate the exactness of the analytical methods.

January 1916.

STUDIES ON PROTEINS.

II. ON THE CAPACITY OF EGG-ALBUMIN TO COMBINE WITH ACIDS OR BASES.

BY

S. P. L. SÖRENSEN

IN COLLABORATION WITH

MARGRTHE HÖYRUP, JENNY HEMPEL AND S. PALITZSCH.

As mentioned in the introduction to these studies[1]), we consider an ammonium-sulphate-containing egg-albumin solution as being a two-phased system in which the dispersion medium is represented by the ammonium sulphate solution, while the disperse phase — generally, by way of abbreviation, designated "Egg-hydrate" — consists of egg-albumin more or less loosely connected with greater or smaller quantities of the components of the dispersion medium.

If small amounts of sulphuric acid or ammonia are added to such an egg-albumin solution, not only the hydrogen-ion concentration of the solution will be altered, but also other properties of the solution, such as for instance the viscosity and the osmotic pressure. This alteration of properties is closely connected with the distribution of the added quantity of sulphuric acid or ammonia between the two phases of the solution, and it is therefore of great interest to study this distribution more closely.

In the present paper we have to deal with investigations of this kind and intend more especially to answer the following questions: —

Is it possible, by measuring the hydrogen-ion concentration of an ammonium sulphate containing egg-albumin solution with known contents of protein nitrogen and ammonia nitrogen,

1) *to determine the whole amount of "surplus" sulphuric acid or ammonia present*, and 2) *to determine the amount of "surplus"*

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 8 , (1915).

sulphuric acid or ammonia combined with the egg-albumin? By "surplus" sulphuric acid we mean the amount of sulphuric acid existing in the solution beyond the quantity equivalent to the amount of ammonia present.

In the discussion of these questions we have regarded the two-phased system occurring in egg-albumin solutions in exactly the same way as the common homogeneous systems; in other words, as a real solution of a very complicated amphoteric compound; and we have tried by comparison of the behaviour of egg-albumin solutions with that of simple ampholyte solutions to find some support for such a view. Further, we have in our considerations pre-supposed that the hydrogen-ion concentration of an ampholyte solution depends entirely on the quantity and the degree of dissociation of the part of the surplus acid or base not combined with the ampholyte. Thus we have assumed, in the case of egg-albumin solutions, that the hydrogen-ion concentration of the solution depends solely on the composition of the dispersion medium, and not on the concentration of the disperse phase or the composition of the latter. This assumption is not quite correct, but is subject to an error (the nature and significance of which will be thoroughly dealt with in the following); but the assumption referred to will enable us to give an answer to the questions above made which in the majority of cases is very nearly correct. This answer is based upon a series of hydrogen-ion measurings of ammonium-sulphate-containing egg-albumin solutions of known composition and known contents of surplus sulphuric acid or ammonia[1]).

Suppose, by way of explanation, 100 cc. of such an egg-albumin solution of known composition to contain, for instance, t cc. $^n/_{1000}$ surplus sulphuric acid, and suppose further the hydrogen-ion measurement of the solution to give such a value that it will be possible in the manner described in the following Section A to calculate that the ammonium sulphate solution, the dispersion medium, in 100 cc. experimental liquid contains t' cc. $^n/_{1000}$ surplus sulphuric acid. It is then directly evident that the egg-albumin, the disperse phase, in the 100 cc. experimental liquid is combined with $t \div t'$ cc. $^n/_{1000}$ surplus sulphuric

[1]) The preparation of such solutions, the composition of which is exactly known, is thoroughly explained in our Paper I (Comptes-rendus du Laboratoire de Carlsberg, **12**, 25 (1916).

acid. If now 100 cc. experimental liquid contains e milligram-equivalents protein-nitrogen, the *capacity to combine with acids will be* $\frac{t \div t'}{e}$, *expressed as the number of cubic centimetres* n/1000 *surplus sulphuric acid combined with one milligram-equivalent protein nitrogen*[1]).

By numerous experiments of this kind it is shown that the combining capacity for sulphuric acid or ammonia, as was to be expected, is altered with the hydrogen-ion concentration, while, as might also have been expected, it may be commonly considered as independent of the protein concentration. Further, the combining capacity has proved to be slightly dependent on the ammonium sulphate concentration, which fact agrees perfectly with the theory (see p. 106). *The dependence of the combining capacity on the hydrogen-ion concentration and the ammonium sulphate concentration may be represented graphically in a very perspicuous manner.*

The results thus obtained, and especially their graphical representation, may now be used in answering the above questions, which form the subject-matter of this paper. The fact is that it is possible by means of the graphical representation just mentioned, to determine the amount of surplus sulphuric acid or ammonia which is combined with the protein in an ammonium sulphate containing egg-albumin solution with known contents of protein nitrogen and ammonia nitrogen, provided that the hydrogen-ion concentration of the solution has been measured. Since it is further possible (as mentioned p. 69) to calculate the content of surplus sulphuric acid or ammonia in the ammonium sulphate solution if the hydrogen-ion concentration is known, it will be seen that we have at our disposal all data necessary for answering the two above questions.

In the following pages there will in *Section A* first be rendered an account of the theoretical foundation upon which is based the calculation of the content of surplus acid or base of a salt solution by means of the hydrogen-ion concentration of the solution, and then the theory set forth will apply to the salt solu-

[1]) Whether the egg-albumin, besides surplus sulphuric acid, is also combined with some ammonium sulphate, does not appear from the experiments described in this paper, which solely concern the amount of *surplus* sulphuric acid or ammonia.

tion with which we are specially concerned here, namely ammonium sulphate solutions.

In *Section B* the theory of the capacity of ampholytes to combine with acids or bases is discussed, and we try by examples to prove that in investigations into the combining capacity of ampholytes it is permissible, within wide limits, to start on the above assumption (p. 69), *viz.* that the hydrogen-ion concentration of the solution depends solely on the composition of the dispersion medium or, in other words, depends exclusively on the concentration and degree of dissociation of that part of the acid which is not combined with the ampholyte.

Section C deals with the measurements of hydrogen-ion concentrations in egg-albumin solutions of known composition and known content of surplus sulphuric acid or ammonia; the results thus obtained, briefly outlined above, are set forth in greater detail, their application being also explained.

Further it is shown, that the capacity of the egg-albumin to combine with acids or bases may be easily and naturally accounted for from the same points of view as those used in Section B in the case of ampholytes of simple composition.

Finally we shall in *Section D* mention the determination of the isoelectric point of the egg-albumin by means of the experimental material given in the preceding sections.

The paper is completed with a table (Table 32) which, for ammonium sulphate solutions with a certain content, S, of ammonium sulphate per 100 g. water, states the specific gravity, d_s, the equivalent concentration, c, and the volume, V_s, of that weight of the solution which contains 100 g. water. The table, which is used on several occasions both in the present and in the following papers, is elaborated on the basis of some determinations — made by Mr. S. Palitzch and Mr. H. Jessen-Hansen — of the specific gravity of ammonium sulphate solutions, which will be mentioned in a further paper together with the determinations of the specific gravity of egg-albumin solutions.

A. The Connection between the Hydrogen-ion Concentration of a Salt Solution and its Content of Surplus Acid or Base.

a. Theoretical Basis of the Calculation.

It is well-known, that a *strong* acid, HAc, if sufficiently diluted, may be considered completely dissociated, so that the hydrogen-ion concentration is equal to the acid concentration. If a salt of the acid and a *strong* base, MOH, is added, the dissociation of the acid will be pushed back, and the hydrogen-ion concentration, h, will only be a fractional part of the acid concentration, s:

$$h = \alpha \cdot s \qquad (1).$$

As the quantity α, that is the degree of dissociation of the acid, is contingent upon the concentration of the anion, $Ac^{\div}$, which is common to the acid and the salt, α will be a constant if $[Ac^{\div}]$ is a constant, which will be the case when the concentration of the salt is constant and when, in proportion to the acid concentration, it is so great that the quantity of the anions formed by the dissociation of the acid, is minimal as compared with those originating in the salt. If h is plotted as abscissa against s as ordinate, Equation (1) will represent a straight line through the point of origin[1]).

It is easily seen that, as far as salt solutions with surplus base are concerned, considerations can be applied which are quite analogous to those we have used here and later on in the treatment of salt solutions with surplus acid.

The matter is somewhat more complicated, when dealing with a salt of the acid with a *weak* base, in which case the hydrolysis of the salt:

$$MAc + H_2O \rightarrow MOH + HAc$$

---

[1]) If $s = 0$, Formula (1) gives $h = 0$, which result does not agree with the real state of things, because the hydrogen-ion concentration in aqueous solutions can never be 0. The disagreement arises from the fact that Formula (1) leaves out of consideration the hydrogen-ions which originate in the dissociation of the water. As, however, the hydrogen-ion concentration originating in this source can be, at most, equal to the hydroxyl-ion concentration of the solution, and as the latter, even at so low a hydrogen-ion concentration as $10^{\div 6}$, does not even amount to 1 % of the whole hydrogen-ion concentration,

$$[OH^{\div}] = \frac{k_w}{[H^+]} = \frac{0.72 \cdot 10^{\div 14}}{10^{\div 6}} = 0.72 \cdot 10^{\div 8},$$

the source of error here mentioned will be of no significance at hydrogen-ion concentrations of the magnitude $10^{\div 6}$ and upwards.

may be of significance for the magnitude of the hydrogen-ion concentration.

Let the dissociation constant of the weak base be k_m, the hydrolysis constant of the salt k_{mh} and the dissociation constant of the water k_w, and we shall, under certain assumptions, have

$$k_{mh} = \frac{k_w}{k_m}.$$

The fact is that the equation corresponding with the hydrolysis is:

$$[\mathrm{MOH}]\ [\mathrm{HAc}] = k_{mh}\ [\mathrm{MAc}],$$

and that corresponding with the electrolytic dissociation of the weak base:

$$[\mathrm{M^+}]\ [\mathrm{OH^-}] = k_m\ [\mathrm{MOH}],$$

These equations give:

$$k_{mh} = \frac{[\mathrm{M^+}]\ [\mathrm{OH^-}]\ [\mathrm{HAc}]}{k_m\ [\mathrm{MAc}]} \qquad (2).$$

Suppose now that HAc and MAc are dissociated to the same degree; then we may put $[\mathrm{HAc}] = r\ [\mathrm{H^+}]$ and $[\mathrm{MAc}] = r\ [\mathrm{M^+}]$, so that Equation (2) will assume the form

$$k_{mh} = \frac{[\mathrm{M^+}]\ [\mathrm{OH^-}]\ r\ [\mathrm{H^+}]}{k_m \cdot r\ [\mathrm{M^+}]} = \frac{k_w}{k_m}.$$

However, this value of k_{mh}, which is commonly used, is not quite correct, because the assumption mentioned is not quite exact even in the case of the strongest acids, and in that of medium-strong or weak acids we should by no means be justified in reckoning with the same degree of dissociation of the acid and the salt. Here we will put the degree of dissociation of the acid equal to α, that of the salt equal to β, the equivalent concentration of the salt equal to c, and the concentration of the acid formed by the hydrolysis (both the dissociated and the undissociated part of it) equal to x. Then Equation (2) will get the form:

$$k_{mh} = \frac{[\mathrm{M^+}]\ [\mathrm{OH^-}]\ x\ (1-\alpha)}{k_m \cdot c\ (1-\beta)},$$

and as $[\mathrm{H^+}] = x \cdot \alpha$ and $[\mathrm{M^+}] = c \cdot \beta$, we shall have

$$k_{mh} = \frac{c \cdot \beta \cdot [\mathrm{OH^-}]\ [\mathrm{H^+}]\ (1-\alpha)}{k_m \cdot c \cdot \alpha\ (1-\beta)} = \frac{k_w \cdot \beta\ (1-\alpha)}{k_m \cdot \alpha\ (1-\beta)} \qquad (3).$$

Hence the hydrolysis constant depends not only on the dissociation constant of the weak base (k_m), but also on the degrees of dissociation of the acid and of the salt, that is, on the nature of the acid and the concentration of the salt.

Now we will calculate the concentration, x, of the acid formed by hydrolysis in a solution of the salt MAc, where the acid, HAc, is considerably stronger than the base, MOH, and where so much acid has been added to the salt solution that

the concentration of the surplus acid is s. We suppose that the base formed by hydrolysis, the concentration of which, of course, is also equal to x, is not dissociated at all, while the degree of dissociation of the acid, as above, is put equal to α, that of the salt equal to β and the concentration of the salt equal to c.

The hydrolysis equation:

$$[\mathrm{MOH}]\,[\mathrm{HAc}] = k_{mh}\,[\mathrm{MAc}]$$

may then be written

$$x\,(x + s)\,(1 \div \alpha) = k_{mh} \cdot c\,(1 \div \beta),$$

and hence we shall have

$$x = \div \frac{s}{2} \div \sqrt{\frac{s^2}{4} + k_{mh} \cdot c\,\frac{1 \div \beta}{1 \div \alpha}}.$$

Using the value of k_{mh} in Equation (3), we obtain

$$x = \div \frac{s}{2} \div \sqrt{\frac{s^2}{4} + c\,\frac{k_w \cdot \beta}{k_m \cdot \alpha}} \qquad (4).$$

If no acid, but a slight dose of the weak base (for instance of concentration m) has been added to the salt solution, the calculation of the concentration x of the acid formed by hydrolysis will be quite the same, the hydrolysis equilibrium being also in this case:

$$[\mathrm{MOH}]\,[\mathrm{HAc}] = k_{mh}\,]\mathrm{MAc}]$$

or $$(m + x)\,x\,(1 \div \alpha) = k_{mh} \cdot c\,(1 \div \beta),$$

and this equation gives an expression of x corresponding exactly with Equation (4), only with the difference that s is replaced by m.

In (4). only the positive sign before the square-root is applicable, since x is positive only in this case. The equation shows — as might be expected — that x is equal to 0 if $c = 0$, If c is constant, the term $c\,\frac{k_w \cdot \beta}{k_m \cdot \alpha}$ will be constant, and it is self-evident that the latter in proportion to $\frac{s^2}{4}$ will be of the greater significance the less $\frac{s^2}{4}$ is. Therefore, if c is constant, x will be the greater the less s (or m) is, and it will have the *greatest value* (x_{max}) when s (or m) is equal to 0:

$$x_{max} = \sqrt{c\,\frac{k_w \cdot \beta}{k_m \cdot \alpha}} \qquad (5).$$

If k_m, k_w, c, β and α are known, x may then be calculated for any value of s or m, and even if the quantities mentioned

are known only approximately — as may often be the case, especially with β —, it is possible to form a fairly exact estimation of the magnitude of x. Examples of such calculations will be found below (p. 81).

Reverting to Equation (1)

$$h = a \cdot s,$$

we see that in the case here before us — the salt of the acid and a weak base — this equation must be altered into

$$h = a \cdot (s + x) \qquad (6),$$

and only if x is minimal as compared with s, will Equation (6) be identical with Equation (1).

If now the problem to be solved is to determine a by means of measurements of hydrogen-ions in solutions of the salt with known amounts of surplus acid, we may use so great amounts of acid that x is minimal as compared with s, that is, we may — as we have done in the experiments described in the experimental part — calculate a fairly correct value of a from the measurements with the great amounts of acid, and this value is then used for the calculation of x. If x is determined in this manner, it will, of course, be possible, theoretically speaking, to use all the measurements for the calculation of a, even such measurements where s is equal to 0, or where not an acid but a base has been added to the salt solution, the equation

$$h = a \cdot (s + x)$$

being in this case reduced to

$$h = a \cdot x.$$

It will, however, not be suitable to the purpose to attribute to the measurements in experimental mixtures containing surplus base the same value as to the other measurements, because the hydrogen-ion concentration is in this case contingent entirely on the often very small amount of acid formed by the hydrolysis. From a theoretical point of view, however, all the measurements may, as just mentioned, be used for the calculation of a by means of the formula

$$h = a \cdot (s + x),$$

and the above mentioned graphical representation (see p. 72) will, if c is constant and h is plotted as abscissa against $(s + x)$ as ordinate, also in this case give a straight line through the origin.

Instead of α we often use the reciprocal value of this quantity; we designate $\frac{1}{\alpha}$ as f_s which is called the *acid factor*. f_s *being the factor by which the hydrogen-ion concentration,* h, *has to be multiplied in order to give* $(s + x)$, *which is the whole amount of acid present in the solution. both that part of it which is added and that formed by hydrolysis.*

Our aim has hitherto been, by means of measurements of hydrogen-ions, to determine the values of α or f_s corresponding with certain values of the salt concentration, c. The problem may, however, if α and f_s are known, also be the reverse: by means of measurements of hydrogen-ions to determine the whole acid concentration $s + x$ of a solution, in order, by this means and through the use of Equation (4), to be enabled to calculate the *concentration*, s, *of surplus acid of the solution. that is. the acid concentration exhibited by the solution beyond the acid concentration equivalent to the base concentration.*

The calculation is then made in this way:

$$s + x = h \cdot f_s,$$

and Equation (4)

$$x = \div \frac{s}{2} + \sqrt{\frac{s^2}{4} + c \frac{k_w \cdot \beta}{k_m \cdot \alpha}}$$

will by elimination of x give:

$$s = h \cdot f_s \div \frac{c \cdot \frac{k_w \cdot \beta}{k_m \cdot \alpha}}{h \cdot f_s} = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s} \qquad (7).$$

If (7) is written in another way:

$$s = h \cdot f_s \left(1 \div \left(\frac{x_{max}}{h \cdot f_s}\right)^2\right),$$

it will be seen that, if $x_{max} < h \cdot f_s$, s will be positive. If x_{max} is equal to $h \cdot f_s$, s will be equal to 0. which means that the solution contains the acid and the base in equivalent quantities. Finally, if $x_{max} > h \cdot f_s$, s will be negative, that is the solution contains surplus base. The latter result may, of course, also be deduced from the equations relating to solutions with surplus base:

$$x = h \cdot f_s$$

and

$$x = \div \frac{m}{2} + \sqrt{\frac{m^2}{4} + c \frac{k_w \cdot \beta}{k_m \cdot \alpha}}.$$

From these equations we have, as above:

$$m = \div h \cdot f_s + \frac{(x_{max})^2}{h \cdot f_s} = \div h \cdot f_s \left(1 \div \left(\frac{x_{max}}{h \cdot f_s}\right)^2\right).$$

The theoretical considerations above set forth relate to a monobasic acid, and would for instance be decidedly applicable to the case of solutions of ammonium chloride with or without surplus hydrochloric acid or ammonia. In our studies on proteins, however, solutions of ammonium sulphate have played a much more prominent part, and we will now try to apply the theory set forth to such solutions.

If the question is about mixtures in which *the amount of sulphuric acid is small as compared with the amount of ammonium sulphate,* the acid may be considered to be present in the form of an acid salt, HNH_4SO_4, and, generally speaking, we may be warranted in using the same considerations with regard to this acid $H(NH_4SO_4)$ as in the case of the acid HAc considered above.

It is to be borne in mind, however, that while mixtures of the acid HAc and its salt MAc can only give the following ions and undissociated compounds:

$$H^+,\ Ac\div,\ M^+,\ HAc \text{ and } MAc,$$

mixtures of sulphuric acid and ammonium sulphate will be able to form a greater number of ions and undissociated compounds, namely:

$$H^+,\ HSO_4\div,\ SO_4\div\div,\ NH_4^+,\ NH_4SO_4\div,\ H_2SO_4,\ HNH_4SO_4 \text{ and } (NH_4)_2SO_4.$$

It may, however, easily be shown that also in the latter case the rule above laid down will hold good, namely that the acid concentration, s, is equal to the product of the hydrogen-ion concentration, h, and a quantity f_s, which depends on the ammonium sulphate concentration, c, but is otherwise constant.

We will for the present look at it apart from a possible hydrolysis of ammonium sulphate, supposing that the concentration of the ions $NH_4SO_4\div$ and $SO_4\div\div$ only depends on the ammonium sulphate concentration, c, and therefore is constant if c is constant, even if the values of s are varying.

If now we reckon with equivalent concentrations, we shall have

$$s = [H^+] + [HSO_4\div] + [H_2SO_4] + [HNH_4SO_4].$$

If, further, the two dissociation constants of the sulphuric acid are k' and k'', we shall have

$$[HSO_4^-] = \frac{[H^+]\,[SO_4^{--}]}{k''},$$

$$[H_2SO_4] = \frac{[H^+]\,[HSO_4^-]}{k'} = \frac{[H^+]^2\,[SO_4^{--}]}{k' \cdot k''} \text{ and}$$

$$[HNH_4SO_4] = \frac{[H^+]\,[NH_4SO_4^-]}{k_,''}.$$

By substituting these values in the above equation, and replacing $[H^+]$ by h, we get:

$$s = h + h\frac{[SO_4^{--}]}{k''} + h^2\frac{[SO_4^{--}]}{k'\,k''} + h\frac{[NH_4\,SO_4^-]}{k_,''}$$

$$s = h\left(1 + \frac{[SO_4^{--}]}{k''}\left(1 + \frac{h}{k'}\right) + \frac{[NH_4\,SO_4^-]}{k_,''}\right).$$

The values of s here considered are so small that h will always be inferior to 10^{-4}, and as k' is[1]) about $4.5 \cdot 10^{-1}$, $\frac{h}{k'}$ will be minimal as compared with 1; accordingly, the equation may be reduced to:

$$s = h\left(1 + \frac{[SO_4^{--}]}{k''} + \frac{[NH_4\,SO_4^-]}{k_,''}\right).$$

The quantity in parenthesis is, however, under the given assumptions constant, if c is kept constant; that is, the equation

$$s = h \cdot f_s$$

is applicable also in this case.

It is to be noted that the quantity which in the above calculation is discarded as insignificant corresponds with the concentration of undissociated sulphuric acid, H_2SO_4. Consequently, in such solutions as those here in question we may leave out of consideration the contents of undissociated sulphuric acid, which means that mixtures of ammonium sulphate and comparatively small amounts of sulphuric acid behave as mixtures of ammonium sulphate and acid ammonium sulphate, which mixture can directly give all the ions and undissociated compounds that are stated p. 77, with the sole exception of H_2SO_4.

As regards the question as to the significance of the hydrolysis of the ammonium sulphate, we must bear in mind that it can be of consequence only in the case of solutions very poor in acid, and with hydrogen-ion concentrations considerably below

[1]) Karl Jellinek: Zeitschr. physik. Chem. **76**, 331 (1911).

the above mentioned value $10^{\div 4}$. It is therefore impossible that measurable quantities of H_2SO_4 could be formed by the hydrolysis; the hydrolysis will only reach as far as the first phase:

$$(NH_4)_2SO_4 + H_2O \rightarrow NH_4OH + HNH_4SO_4,$$

which corresponds exactly with the general hydrolysis form (mentioned on p. 72)

$$MAc + H_2O \rightarrow MOH + HAc,$$

and may be treated in the same way.

If a quite similar calculation as that above stated (p. 74) is made, and if c signifies the equivalent concentration of the ammonium sulphate, while β_1 and β_2 designate the degrees of dissociation in the dissociation of the first and the second ammonium group of the ammonium sulphate, respectively, it will be found that

$$x = \div \frac{s}{2} \div \sqrt{\frac{s^2}{4} + c \frac{k_w \frac{\beta_1}{2}(1 + \beta_2)}{k_m \cdot a}},$$

and if here $\frac{\beta_1}{2}(1 + \beta_2)$ is put equal to β, the expression will be identical with that obtained above (Equation (4)).

Thus, if c. k_w, k_m, a and β are known, the Equation (4) may be employed for the calculation of x, if s has a given value, just as Equation (7)

$$s = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s}$$

may be used for the calculation of s if the other quantities are known or can be measured.

As for the above mentioned "known" quantities, c means, as we have just said, the equivalent concentration of the ammonium sulphate; k_w is put equal to $0.72 \cdot 10^{\div 14}$ (18^0)[1]), k_m equal to $1.75 \cdot 10^{\div 5}$ (18^0)[2]); f_s may for a given value of c be read off on the curve given in the experimental part (Fig. 4, p. 89), and a is equal to $\frac{1}{f_s}$. Before this curve was constructed, we were, however, as described above (p. 75), able in another way to arrive at a nearly correct value of a.

As to β, the case was much more difficult, because we know only very little about its variation with the ammonium sulphate

[1]) Comptes-rendus du Laboratoire de Carlsberg, **8**, 31 (1909).

[2]) H. Lundén: Meddelanden från K. Vetensk.-Akad. Nobelinstitut, **1**, Nr. 8, p. 5 (1907). A. A. Noyes, Yogoro Kato and R. B. Sosman: Zeitschr. physik. Chem. **73**, 7 (1910).

concentration. Considering, however, that the dissociation of the ammonium chloride and that of the potassium chloride vary in quite the same way with the concentration, it is an obvious conclusion that the dissociation of the ammonium sulphate will depend on the concentration in a similar manner to that of the potassium sulphate. By means of the determinations of conductivity made by Winston and Clover[1]) and those made by Kohlrausch and Klein[2]) of weak and of strong solutions of ammonium sulphate, respectively, as compared with the determinations of conductivity of potassium sulphate solutions, we have estimated the variation of the equivalent conductivity of ammonium sulphate solutions with the concentration, and from this we have estimated the variation of β. By this estimation we have assumed that the dissociation of the second ammonium group does not begin until the dissociation of the first is completed. We know very well that the values of β found in this way are uncertain; they permit, however, as will appear from the examples given in the experimental part, an applicable calculation of x.

In the following Table 7 a general view is given of the values of f_s, β and $\frac{\beta}{a}$ for different values of c.

TABLE 7.

c	f_s[3])	β	$\frac{\beta}{a} = \beta \times f_s$
0.01	1.40	about 0.70	about 0.98
0.02	1.65	,, 0.61	,, 1.01
0.05	2.30	,, 0.49	,, 1.13
0.1	2.72	,, 0.46	,, 1.25
0.2	3.50	,, 0.42	,, 1.47
0.5	5.02	,, 0.37	,, 1.86
1.0	6.70	,, 0.33	,, 2.21
2.0	9.44	,, 0.29	,, 2.74
3.0	11.87	,, 0.27	,, 3.20
4.0	13.87	,, 0.25	,, 3.47
5.0	15.40	,, 0.23	,, 3.54

[1]) Harry C. Jones: The electrical conductivity, dissociation, and temperature coefficients of conductivity, Washington 1912, p. 33.

[2]) See Kohlrausch und Holborn: Das Leitvermögen der Elektrolyte, Leipzig 1898, p. 151.

[3]) The values of f_s are found by means of the curve Fig. 4, p. .

In order to elucidate the significance of the hydrolysis and its dependence on the ammonium sulphate concentration, c, we have in Table 8 set forth the values of x calculated for different values of s and three different concentrations of ammonium sulphate.

TABEL 8.

Equivalent-concentration, s, of surplus acid $s \cdot 10^6$	Equivalent-concentration, x, of acid formed by hydrolysis $x \cdot 10^6$	x in % of s	Hydrogen-ion concentration, h, calculated from the formula $h = \frac{s + x}{f_s}$ $h \cdot 10^6$
$c = 4.0$; $f_s = 13.87$; $c \frac{k_w \cdot \beta}{k_m \cdot \alpha} = 5705 \cdot 10^{\div 12}$			
1000	5.67	0.57	72.51
500	11.16	2.23	36.85
200	25.32	12.66	16.25
100	40.58	40.58	10.14
50	54.56	109.12	7.54
0	75.53	—	5.45
$c = 1.0$; $f_s = 6.70$; $c \frac{k_w \cdot \beta}{k_m \cdot \alpha} = 908 \cdot 10^{\div 12}$			
500	1.81	0.36	74.90
300	3.00	1.00	45.22
200	4.44	2.22	30.51
100	8.38	8.38	16.18
50	14.15	28.30	9.57
0	30.13	—	4.50
$c = 0.1$; $f_s = 2.72$; $c \frac{k_w \cdot \beta}{k_m \cdot \alpha} = 51 \cdot 10^{\div 12}$			
200	0.26	0.13	73.62
100	0.51	0.51	36.95
50	1.00	2.00	18.75
20	2.29	11.45	8.19
0	7.14	—	2.62

b. Experimental part: Determination of the Acid Factor, f_s.

If, in a series of ammonium sulphate solutions of equal concentration, c, but to which different quantities of a very diluted sulphuric acid have been added, the hydrogen-ion concentration, h, is measured, the theory above set forth (see p. 78) demands a graphical sketch of the results of the experiments to be a straight line through the origin, if the amounts of sulphuric acid added — expressed for instance in cc. $^{n}/_{1000}$ acid — are plotted as ordinates against the values of h as abscissae, and if the concentration of the acid is of such a value, that the hydrolysis of the ammonium sulphate is of no consequence.

Such experiments have, however, proved that the straight line which graphically shows the results of the experiments, generally does not pass through the origin. The reason is that the ammonium sulphate hardly ever contains ammonia and sulphuric acid in absolutely equivalent quantities; on the contrary it contains a little excess of one of the components, an excess which, however, seldom comes to more than $^{1}/_{50000}$ of the whole quantity. Yet this excess (the nature and quantity of which depend on the way in which the ammonium sulphate has been prepared and recrystallised) is sufficient for displacing the situation of the straight line mentioned, and it is obvious that an amount of a cc. $^{n}/_{1000}$ surplus ammonia in the used quantity of ammonium sulphate solution will be able to displace the straight line without changing its direction, in such a way that the point of intersection with the axis of ordinate will be at $+a$, while on the contrary an amount of a cc. $^{n}/_{1000}$ surplus sulphuric acid will give the point of intersection with the axis of ordinate at $\div a$.

Therefore it is possible from the point of intersection of the straight line with the axis of ordinate to estimate the quantity of surplus ammonia or sulphuric acid contained in the solution of ammonium sulphate used for the experiments. Of course this quantity must enter into the calculation of f_s, which — as just said (see p. 76) — *is the factor by which the hydrogen-ion concentration, h, has to be multiplied in order to give the surplus sulphuric acid concentration, s.* This calculation may, as explained above (see p. 75 and 76), be done simply after the equation:

$$f_s = \frac{s}{h},$$

if only such experiments are used for the calculation in which

the hydrogen-ion concentration is so great, that the hydrolysis is of no consequence. If the measurements of the acid-poor solutions are also required for calculation, it is necessary to take into account the acid concentration, x, formed by the hydrolysis, where x may be found from the equation

$$x = \div \frac{s}{2} + \sqrt{\frac{s^2}{4} + c \frac{k_w \cdot \beta}{k_m \cdot \alpha}}$$

and subsequently f_s from the equation

$$f_s = \frac{s + x}{h}.$$

In all our experiments aiming at the determination of f_s, the hydrolysis has entered into the calculation; yet we have of course, as the detailed description, given below, of a few series of experiments shows, in the graphical representation only taken into account the experiments with the acid-richest mixtures for the determination of the situation of the straight line by means of which the original contents of surplus sulphuric acid or ammonia of the solution of ammonium sulphate can be determined. In the following calculation of f_s, however, we have — paying regard to the hydrolysis — also taken into consideration the results obtained by the acid-poor mixtures. In other measurements, in which we have considered f_s as known, and observed h in order to determine s, we have of course been forced to take the hydrolysis into consideration as well.

In the series of experiments the results of which are set forth in Table 9, we used for each sample 30 cc. ammonium sulphate solution, which was diluted to 50 cc. with the quantities of acid stated in the first column of the table and with water. The hydrogen-ion concentration was measured with three different electrodes; the averages of the values of h are set forth in the second column of the table. If now the figures in the first column — expressed in cc. $^n/_{1000}$ sulphuric acid — are plotted as ordinates against the values of h as abscissae, as done in Fig. 3, it is possible to draw a straight line (in Fig. 3 marked I) through the points corresponding with the acid richest mixtures. This straight line intersects the axis of ordinate in the point + 6.0, from which fact we conclude that the 30 cc. ammonium sulphate solution used for each experimental mixture contain 6 cc. $^n/_{1000}$ surplus ammonia. In the figure it is further seen,

TABLE 9.

30 cc. of a 7.116 · n solution of ammonium sulphate (V) diluted with water and sulphuric acid to 50 cc.; concentration of ammonium sulphate $c = {}^3/_5 \cdot 7.116 = 4.27 \cdot n$. The 30 cc. ammonium sulphate solution was graphically (see Fig. 3) found to contain 6 cc. $^n/_{1000}$ surplus ammonia.

Sulphuric acid added cc.	Hydrogen-ion concentration, h, observed $h \cdot 10^6$	Equivalent-concentration, s, of surplus sulphuric acid $s \cdot 10^6$	$\frac{s}{h}$	Equivalent-concentration, x, of sulphuric acid, formed by hydrolysis $x \cdot 10^6$	$f_s = \frac{s+x}{h}$
10 cc. $^n/_{1000}$	9.04	80	8.85	47.3	14.08
15 ,, ,,	14.18	180	12.69	28.8	14.72
20 ,, ,,	20.28	280	13.81	20.1	14.80
2.5 ,, $^n/_{100}$	27.73	380	13.70	15.2	14.25
3 ,, ,,	34.56	480	13.89	12.2	14.24
3.5 ,, ,,	40.98	580	14.15	10.2	14.40
4 ,, ,,	49.04	680	13.87	8.7	14.04
				Average...	14.36

that the points corresponding to the acid-poor mixtures are not situated on the straight line, as h attains too high a value on account of the sulphuric acid formed by the hydrolysis, the influence of which makes itself felt more and more as the sulphuric acid concentration decreases.

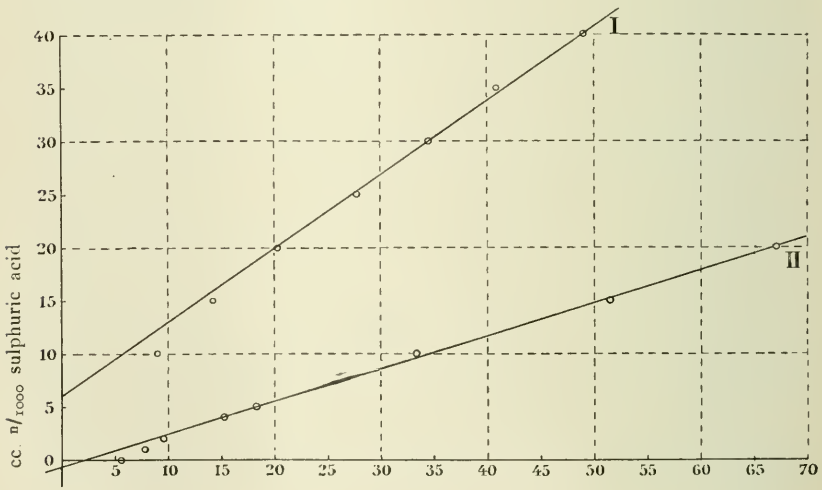

Fig. 3.

Making due allowance for the 6 cc. $^{n}/_{1000}$ surplus ammonia in the solution of ammonium sulphate, we are able to calculate the real surplus sulphuric acid concentration, s, of the experimental mixtures; s is given in the third column of Table 9. In the fourth column the values of f_s. found by division of s by h, are given, and from the table it is seen that while with this method of calculation, which ignores the hydrolysis, the values found in the case of the acid-poor experimental mixtures are too small, the acid-rich mixtures give very nearly the correct values of f_s.

In the calculation of x (the concentration of the sulphuric acid formed by the hydrolysis), the equation:

$$x = \div \frac{s}{2} + \sqrt{\frac{s^2}{4} + c \cdot \frac{k_w \cdot \beta}{k_m \cdot \alpha}},$$

was used, in which we put $c \cdot \frac{k_w \cdot \beta}{k_m \cdot \alpha}$ equal to $6023 \cdot 10^{\div 12}$, using for $\frac{1}{\alpha} = f_s$ the value 14.3[1]) and for β the value 0.24.

The values of x are stated in the fifth column of the table. The decreasing influence of the hydrolysis with increasing acid concentration appears clearly from these figures. Finally, the values of $f_s = \frac{s + x}{h}$ are given in the last column of the table.

Table 10 is to be interpreted in quite the same way. It gives the results of a series of experiments with lower ammonium sulphate concentration, from which reason the hydrolysis acquires an essential importance only at lower acid concentrations than in the preceding experiment. That the hydrolysis is here also of consequence at sufficiently low acid concentrations, is, however, seen both from the columns 4 and 5 of the table and from the graphical sketch, Fig. 3 II, which is to be understood in the same way as Fig. 3 I, and from which it is seen that the 25 cc. solution of ammonium sulphate used for each experimental mixture contains 0.7 cc. $^{n}/_{1000}$ surplus sulphuric acid.

At the bottom of Table 10 a few measurements are set forth in which ammonia was added to the experimental mixture; in

[1]) A preliminary calculation of x with the value 14.0 for $\frac{1}{\alpha} = f_s$ (the average of the last two values of $\frac{s}{h}$) gave the value 14.3 for $\frac{s+x}{h}$; the value 14.3 for $\frac{1}{\alpha}$ was therefore used in the final calculation.

TABLE 10.

25 cc. of a 1.938 · n ammonium sulphate solution (II) diluted with water and sulphuric acid to 50 cc.; ammonium sulphate concentration $c = 0.969 \cdot n$.

Graphically (see Fig. 3) it was found that the 25 cc. ammonium sulphate solution contained 0.7 cc. $^n/_{1000}$ surplus sulphuric acid.

Sulphuric acid added cc.	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Equivalent-concentration, s, of surplus sulphuric acid $s \cdot 10^6$	$\frac{s}{h}$	Equivalent-concentration, x, of sulphuric acid, formed by hydrolysis $x \cdot 10^6$	$f_s = \frac{s+x}{h}$
0 cc. $^n/_{1000}$	5.47	14	2.56	22.2	6.62
1 ,, ,,	7.71	34	4.41	16.1	6.50
2 ,, ,,	9.62	54	5.61	12.2	6.88
4 ,, ,,	15.28	94	6.15	7.9	6.67
5 ,, ,,	18.46	114	6.18	6.7	6.54
10 ,, ,,	33.34	214	6.42	3.7	6.53
15 ,, ,,	51.52	314	6.09	2.5	6.14
20 ,, ,,	67.14	414	6.17	1.9	6.19
				Average . . .	6.51
		$m \cdot 10^6$			$\frac{x}{h}$
2 cc. $^n/_{1000}$ ammonia	3.60	26	—	18.2	5.06
5 cc. $^n/_{1000}$ ammonia	1.70	86	—	8.5	5.00

this case the sulphuric acid formed by the hydrolysis is calculated by means of the formula

$$x = \div \frac{m}{2} + \sqrt{\frac{m^2}{4} + c \frac{k_w}{k_m} \cdot \frac{\beta}{\alpha}},$$

where m is the concentration of surplus ammonia; f_s has been put equal to $\frac{x}{h}$ (see p. 75). The Table shows, that although the value of f_s is somewhat low, still it is of the right order even in these rather uncertain measurements.

We have executed, in all, 25 such series of experiments with ammonium sulphate solutions of different concentration, for the preparation of which 7 samples of ammonium sulphate were

used. Table 11 gives information as to the quality of these samples, and also — in the two last columns — the values found for f_s.

TABLE 11.

Values of f_s found with different concentrations of ammonium sulphate, c.

Ammonium sulphate		Contents of surplus sulphuric acid or ammonia (graphically found, see Fig. 3)	Ratio equivalents of surplus sulphuric acid or ammonia to equivalents of ammonium sulphate	Values of f_s found with different concentrations of ammonium sulphate, c	
Mark	Sort			c	f_s
I	C. A. F. Kahlbaum "Zur Analyse" 1912	30 cc. of a 7.819 · n solution contained 3.0 cc. $n/_{1000}$ surplus ammonia	1 : 78000 (ammonia)	4.692 3.128 1.564 0.782	14.60 11.67 7.73 6.19
II	*Another sample* of the same preparation dissolved four times in water; the solutions precipitated with alcohol	25 cc. of a 1.938 · n solution contained 0.7 cc. $n/_{1000}$ surplus sulphuric acid	1 : 69000 (sulphuric acid)	0.969	6.51
III	*A third sample,* dissolved twice in water; the solutions precipitated with alcohol	25 cc. of a 1.968 · n solution contained 0.8 cc. $n/_{1000}$ surplus ammonia	1 : 61500 (ammonia)	0.984 0.787 0.590 0.394 0.197 0.064	6.77 6.07 5.40 4.04 3.22 2.23
IV	C. A. F. Kahlbaum "Zur Analyse, mit Garantieschein" 1913	30 cc. of a 6.889 · n solution contained 3.0 cc. $n/_{1000}$ surplus ammonia	1 : 69000 (ammonia)	4.133 3.445 2.756 2.067 1.378 0.689 0.344 0.207 0.069 0.014	13.68 12.88 11.97 10.41 8.56 6.05 4.75 3.62 2.31 1.55
V	Same preparation 1914	30 cc. of a 7.116 · n solution contained 6 cc. $n/_{1000}$ surplus ammonia	1 : 35500 (Ammoniak)	4.270	14.36

Ammonium sulphate		Contents of surplus sulphuric acid or ammonia (graphically found, see Fig. 3)	Ratio equivalents of surplus sulphuric acid or ammonia to equivalents of ammonium sulphate	Values of f_s found with different concentrations of ammonium sulphate, c	
Mark	Sort			c	f_s
VI	C. A. F. Kahlbaum "Zur Analyse mit Garantieschein" 1914 a special sample	30 cc. of a 6.80 · n solution contained 11.8 cc. $^n/_{1000}$ surplus ammonia	1 : 17300 (ammonia)	4.080	13.89
VII	C. A. F. Kahlbaum "Zur Analyse" 1914	30 cc. of a 7.268 · n solution contained 2.2 cc. $^n/_{1000}$ surplus sulphuric acid	1 : 99000 (sulphuric acid)	4.361 2.907	14.70 11.57

The results are graphically set forth in Fig. 4, where the concentration of ammonium sulphate, c, is plotted as ordinate against the value of f_s as abscissa.

By means of the curve thus constructed it is now possible to find f_s for every value of c, and hence we are able to calculate s in a solution of ammonium sulphate when h is measured. If h is sufficiently high ($15 \cdot 10^{-6}$ or more), an approximately correct value of s will be obtained from the simple formula

$$s = h \cdot f_s;$$

but at lower hydrogen-ion concentrations this formula is inapplicable, as it does not take into account the hydrolysis of the ammonium sulphate. In all cases we have taken the hydrolysis into consideration, and used the more complicated formula

$$s = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s},$$

where

$$x_{max} = \sqrt{c \frac{k_w \cdot \beta}{k_m \cdot \alpha}}$$ (see p. 74).

In order to illustrate the exactness of such a measurement of h and the ensuing calculation of s, the results of some mea-

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Concentration of ammonium sulphate, c.
0
2
4
6
8
10
12
14
16
Acid factor, f_s.

Fig. 4.

surements of ammonium sulphate solution VII are given in Table 12.

TABLE 12.

30 cc. of a $7.268 \cdot n$ ammonium sulphate solution (VII) diluted with water and sulphuric acid to 50 cc.; $c = 4.361 \cdot n$; $f_s = 14.5$ (see Fig. 4). The 30 cc. ammonium sulphate solution contained 2.2 cc. $n/_{1000}$ surplus sulphuric acid.

$(x_{max})^2 = 6140 \cdot 10^{\div 12}$.

Sulphuric acid added cc.	Amount of surplus sulphuric acid in the used 30 cc. solution of ammonium sulphate cc.	Equivalent-concentration, s, of the whole surplus sulphuric acid $s \cdot 10^6$ (given)	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Equivalent-concentration, s, of surplus sulphuric acid calculated from h $s \cdot 10^6$ (found)	Difference s (given) $\div$ s (found)
0 cc. $n/_{1000}$	2.2 cc. $n/_{1000}$	44	6.94	39.6	$4.4 \cdot 10^{\div 6}$
5 ,, ,,	,, ,,	144	12.04	139.5	4.5 ,,
10 ,, ,,	,, ,,	244	17.96	236.7	7.3 ,,
15 ,, ,,	,, ,,	344	24.52	338.1	5.9 ,,
20 ,, ,,	,, ,,	444	31.52	443.8	0.2 ,,

Concerning the easily intelligible figures given in the table it may only be remarked that the third column contains the given acid-concentration, *viz.* the concentration of the sum of the amount of acid present in the solution of ammonium sulphate and the added amount of acid, while the fifth column contains the acid concentration calculated by means of the above formula, from the measured hydrogen-ion concentration, h. In the last column of the table the differences between the given and found values of the acid concentration are set forth.

The differences between the given and found acid concentrations will generally be of similar magnitude to those set forth in Table 12, when dealing with investigations of solutions of ammonium sulphate containing surplus sulphuric acid, even in minimal amounts. In all our control experiments the differences seldom reached the magnitude $10 \cdot 10^{\div 6}$, and only in a single instance did they exceed $20 \cdot 10^{\div 6}$. Expressed in % of the whole surplus acid concentration the difference will often seem to be considerable. But only the absolute extent of the difference is here of any interest. Expressed in this way a difference of $20 \cdot 10^{\div 6}$ means an error of 1 cc. $n/_{1000}$ for 50 cc. experimental mixture, and we consider it justifiable to reckon with this difference as

maximum error in investigations of solutions of ammonium sulphate with surplus sulphuric acid.

When we have to deal with solutions without surplus sulphuric acid or with surplus ammonia, that is, solutions with a hydrogen-ion concentration less than about $6 \cdot 10^{\div 6}$, the case is different. The measurement of the hydrogen-ion concentration is in this case, as before mentioned (p. 75), less exact, and in the calculation of s, which in the cases mentioned must be 0 or negative, the quantity x_{max} is of paramount importance. As, however, x_{max} (especially on account of the uncertain value of β) is subject to essential errors, the *maximum error* in the determination of s in such solutions may be considerably greater than 1 cc. $^{n}/_{1000}$ for 50 cc. experimental mixture. The results found for such solutions may therefore be considered as being only approximately correct. In order to illustrate this, we have in Table 13 put together some measurements executed with some of the solutions of ammonium sulphate used for the determinations of f_s, but without addition of sulphuric acid (in a few experiments with addition of a little ammonia). The contents of surplus sulphuric acid or ammonia in these solutions were known, and could of course also be calculated by means of the hydrogen-ion concentrations measured; by this means a comparison between the given and the found contents was possible. In this case, too, the calculation was made by means of the formula

$$s = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s},$$

where a negative value of s means the corresponding concentration of ammonia, which concentration we have before sometimes designated by m.

It appears from Table 13 (p. 92) that even in such cases as those here considered the difference is generally inferior to $20 \cdot 10^{\div 6}$ (corresponding to 1 cc. $^{n}/_{1000}$ for 50 cc. experimental mixture); it is only in the aqueous solution of ammonium sulphate VI — which contains an abundant amount of surplus ammonia and therefore gives a very low value of h — that the difference is considerably higher.

TABEL 13.

Ammonia added cc.	Amount of surplus sulphuric acid or ammonia in the ammonium sulphate solution used cc.	Equivalent-concentration, s, of the whole surplus sulphuric acid $s \cdot 10^6$ (given)	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Equivalent-concentration, s, of surplus sulphuric acid, calculated from h $s \cdot 10^6$ (found)	Difference s (given) $\div$ s (found)
	25 cc. of a 1.938 · n solution of ammonium sulphate (II) diluted with water and ammonia to 50 cc.; $c = 0.969$; $f_s = 6.6$ (see Fig. 4). The 25 cc. ammonium sulphate solution contained 0.7 cc. $^n/_{1000}$ surplus sulphuric acid. $(x_{max})^2 = 849 \cdot 10^{\div 12}$. [1]				
0 cc. $^n/_{1000}$	0.7 cc. $^n/_{1000}$ H_2SO_4	+ 14	5.47	+ 12.6	+ 1.4 · $10^{\div 6}$
2 ,, ,,	,,	$\div$ 26	3.60	$\div$ 12.0	$\div$ 14.0 ,,
5 ,, ,,	,,	$\div$ 86	1.70	$\div$ 64.4	$\div$ 21.6 ,,
	25 cc. of a 1.968 · n solution of ammonium sulphate (III) diluted with water to 50 cc.; $c = 0.984$; $f_s = 6.65$ (see Fig. 4). The 25 cc. solution of ammonium sulphate contained 0.8 cc. $^n/_{1000}$ surplus ammonia. $(x_{max})^2 = 866 \cdot 10^{\div 12}$.				
0 ,, ,,	0.8 cc. $^n/_{1000}$ NH_3	$\div$ 16	3.57	$\div$ 12.7	$\div$ 3.3 ,,
	30 cc. of a 6.80 · n solution of ammonium sulphate (VI) diluted with water to 50 cc.; $c = 4.080$; $f_s = 14.0$ (see Fig. 4). The 30 cc. solution of ammonium sulphate contained 11.8 cc. $^n/_{1000}$ surplus ammonia. $(x_{max})^2 = 5663 \cdot 10^{\div 12}$.				
0 ,, ,,	11.8 cc. $^n/_{1000}$ NH_3	$\div$ 236	1.33	$\div$ 285	+ 49 ,,
	30 cc. of a 7.268 · n solution of ammonium sulphate (VII) diluted with water to 50 cc.; $c = 4.361$; $f_s = 14.5$ (see Fig. 4). The 30 cc. solution of ammonium sulphate contained 2.2 cc. $^n/_{1000}$ surplus sulphuric acid. $(x_{max})^2 = 6140 \cdot 10^{\div 12}$. [2]				
0 ,, ,,	2.2 cc. $^n/_{1000}$ H_2SO_4	+ 44	6.94	+ 39.6	+ 4.4 ,,

[1]) These three experiments with the solution of ammonium sulphate (II) are set forth in Table 10, only calculated in another way.

[2]) This experiment belongs to the series set forth in Table 12.

Ammonia added cc.	Amount of surplus sulphuric acid or ammonia in the ammonium sulphate solution used cc.	Equivalent-concentration-s, of the whole surplus sulphuric acid $s \cdot 10^6$ (given)	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Equivalent-concentration, s, of surplus sulphuric acid, calculated from h $s \cdot 10^6$ (found)	Difference s (given) $\div$ s (found)
20 cc. of a 7.268 · n solution of ammonium sulphate (VII) diluted with water to 50 cc ; $c = 2.907$; $f_s = 11.65$ (see Fig. 4). The 20 cc. solution of ammonium sulphate contained 1.47 cc. $^n/_{1000}$ surplus sulphuric acid. $(x_{max})^2 = 3686 \cdot 10^{\div 12}$.					
0 cc. $^n/_{1000}$	1.47 cc. $^n/_{1000}$ H_2SO_4	+ 29.4	6.90	+ 34.5	$\div$ 5.1 · $10^{\div 6}$

B. The Capacity of Ampholytes to combine with Acids or Bases.

a. The Hydrogen-ion concentration of salt-free solutions of Ampholytes.

In an acid-containing solution of an ampholyte, RHOH, the hydrogen-ion concentration will be determined exclusively by that part of the acid which is not combined with the ampholyte and by the degree of dissociation of the acid, provided that the concentration of the acid is so great that the dissociation of the ampholyte will be of no consequence. Let the equivalent concentration of the ampholyte be a, the concentration of the added acid s, that of the part of acid not combined with the ampholyte y, and let further the degree of dissociation of the acid be α, that of the salt of the acid together with the ampholyte β', — and we have the usual hydrolysis equilibrium:

$$RHAc + H_2O \rightleftarrows RHOH + HAc$$
$$[HAc] \cdot [RHOH] = k_{bh} [RHAc],$$

which, if $a > s$, gives:

$$y\ (1 \div \alpha)\ (a \div s + y) = k_{bh}\ (s \div y)\ (1 \div \beta'),$$

where k_{bh} is the hydrolysis constant of the salt of the ampholyte.

By a calculation quite similar to that used above (see p. 73) it is possible to find k_{bh}:

$$k_{bh} = \frac{k_w\ (1 \div \alpha)\ \beta'}{k_b\ (1 \div \beta')\ \alpha}.$$

If k_{bh} in the above equation is replaced by this value, we shall by a simple ciphering have

$$y = \div \frac{a \div s + \frac{k_w \cdot \beta'}{k_b \cdot a}}{2} + \sqrt{\frac{\left(a \div s + \frac{k_w \cdot \beta'}{k_b \cdot a}\right)^2}{4} + s \frac{k_w \cdot \beta'}{k_b \cdot a}} \quad (1)$$

This Formula (1) permits the calculation of y and consequently of the hydrogen-ion concentration, h, and of the bound acid concentration, $s \div y$, if the quantities of the right side of the equation are known, and the formula corresponds fairly with the hydrolysis equation given for a salt of a strong acid and a weak base, if an excess of the base has been added to the salt solution:

$$x = \div \frac{m}{2} + \sqrt{\frac{m^2}{4} + c \frac{k_w \cdot \beta}{k_m \cdot a}} \text{ (see p. 74)},$$

where s corresponds with the salt concentration c and $a \div s$ with the concentration of surplus base m (the quantity $\frac{k_w \cdot \beta'}{k_b \cdot a}$ is generally minimal as compared with $a \div s$).

If $s > a$, the hydrolysis equation:

$$[HAc]\ [RHOH] = k_{bh}\ [RHAc],$$

— which, of course, holds good in this case also — will give:

$$(s \div a + y)\ (1 \div \alpha)\ y = k_{bh}\ (a \div y)\ (1 \div \beta').$$

This equation will give an expression for y corresponding exactly with Formula (1), only with the difference that it contains a instead of s and s instead of a; and this equation will correspond with the equation found above (see p. 74)

$$x = \div \frac{s}{2} \div \sqrt{\frac{s^2}{4} + c \frac{k_w \cdot \beta}{k_m \cdot a}}.$$

As before mentioned, Formula (1) is only correct under the assumption that the acid concentration is so great that the dissociation of the ampholyte into hydrogen-ions and ampholyte anions will be of no consequence. In the introductory chapter of this paper (p. 69) it is mentioned that, in the calculation of the capacity of the egg-albumin to combine with acids, we suppose this assumption to be correct; it will now be of some interest to examine, of what magnitude the hydrogen-ion concentration or the concentration of surplus acid must be for this conclusion

to be permissible and, consequently, Formula (1) to be applicable without any material error. We will therefore try to calculate exactly the hydrogen-ion concentration of such acid-containing ampholyte solutions, while taking into account also the dissociation of the ampholyte into hydrogen- and anions and into hydroxyl- and kations, respectively.

Let the concentration of the undissociated ampholyte be u, while the concentration of the ampholyte which is split up into ampholyte-kations and hydroxyl-ions is δ and the concentration of the ampholyte split up into anions and hydrogen-ions is ε. If, for the rest, we use the same designations as above, the different compounds and ions existing in the solution will have the following concentrations:

	[RHOH]	[RH$^+$]	[ROH$^\div$]	[RHAc]
Conc.	u	$(s \div y)\ \beta' + \delta$	ε	$(s \div y)\ (1 \div \beta')$
	[Ac$^\div$]	[HAc]	[H$^+$]	[OH$^\div$]
Conc.	$(s \div y)\ \beta' + \alpha y$	$y\ (1 \div \alpha)$	h	$\frac{k_w}{h}$

These quantities are connected by the following five equations:

(1) The dissociation of the ampholyte as an acid:

$$[H^+]\ [ROH^\div] = k_a\ [RHOH]$$
$$h \cdot \varepsilon = k_a \cdot u$$

(2) The dissociation of the ampholyte as a base:

$$[OH^\div]\ [RH^+] = k_b\ [RHOH]$$
$$\frac{k_w}{h} \cdot ((s \div y)\ \beta' + \delta) = k_b \cdot u$$

(3) The hydrolysis equation of the ampholyte salt:

$$[HAc]\ [RHOH] = k_{bh}\ [RHAc]$$
$$y\ (1 \div \alpha) \cdot u = \frac{k_w\ (1 \div \alpha) \cdot \beta'}{k_b\ (1 \div \beta') \cdot \alpha}\ (s \div y)\ (1 - \beta')$$
$$y \cdot u = \frac{k_w \cdot \beta'\ (s \div y)}{k_b \cdot \alpha}$$

(4) The whole concentration of ampholyte is equal to a:

$$a = u + (s \div y)\ \beta' + \delta + \varepsilon + (s \div y)\ (1 \div \beta')$$
$$a = u + \delta + \varepsilon + s \div y$$

(5) The sum of the positive ions is equal to the sum of negative ions:

$$(s \div y)\,\beta' + \delta + h = \varepsilon + (s \div y)\,\beta' + a \cdot y + \frac{k_w}{h}$$

$$h \div \frac{k_w}{h} = a \cdot y + (\varepsilon \div \delta).$$

If k_a, k_b, k_w, a, β', a and s are known, it will be possible to find the five unknown quantities y, h, u, δ and ε from the above five equations. These may in fact be reduced to the following two equations:

$$s \div y = \frac{a \cdot a \cdot y \cdot h \cdot k_b}{a \cdot y \cdot h \cdot k_b\,(1 \div \beta') + \beta'\,(h^2\,k_b + h\,k_w + k_a\,k_w)} \qquad (2)$$

and

$$h \div \frac{k_w}{h} = a \cdot y + \frac{a \cdot \beta'\,(k_a \cdot k_w \div h^2\,k_b + h \cdot k_b \cdot a \cdot y)}{a \cdot y \cdot h \cdot k_b\,(1 \div \beta') + \beta'\,(h^2 \cdot k_b + h \cdot k_w + k_a \cdot k_w)} \qquad (3)$$

Equation (3) is of the fourth degree with regard to h, but only of the second degree with regard to $a \cdot y$. Therefore it will answer the purpose if we consider h as given and $a \cdot y$ and s as unknown, in other words, if we calculate what value s must have in order to give the ampholyte solution a certain hydrogen-ion concentration. In this calculation we will provisionally put a and β' equal to 1 and then from Equation (3)[1]) calculate y for a given value of h. When y is found, it will be possible to calculate s from Equation (2). From this approximate value of s the correct value of a and β' may be estimated with sufficient exactness, and then a recalculation is made with these values of a and β'.

In order to get an idea as to the magnitude of the error committed in adopting Formula (1), we will now by means of this formula calculate the hydrogen-ion concentration in a series of glycocoll solutions to which small amounts of hydrochloric acid are added, and then we will — by the aid of the complete formula (Equations 2 and 3) — calculate the quantity of hydrochloric acid which is necessary to give such glycocoll solutions

[1]) If a and β' are put equal to 1, it is possible to reduce the two equations to one, which will assume the following form:

$$h^4 + h^3\left(a \div s + \frac{k_w}{k_b}\right) + h^2 \cdot \frac{k_w}{k_b}\,(k_a \div k_b \div s) \div h \cdot \frac{k_w}{k_b}\,(a \cdot k_a + s \cdot k_a + k_w) \div \frac{k_a \cdot k_w^2}{k_b} = 0,$$

and this equation is identical with another equation found in a somewhat different way and published in an earlier paper (S. P L. Sörensen: Ergebnisse der Physiologie, **12**, 504 (1912).

the same hydrogen-ion concentrations. In these calculations we have put $k_w = 0.72 \cdot 10^{\div 14}$, $k_a = 1.12 \cdot 10^{\div 10}$[1]) and $k_b = 1.65 \cdot 10^{\div 12}$[1]). The degree of dissociation of the hydrochloric acid, α, is calculated from the conductivity of highly diluted solutions of hydrochloric acid, μ_∞ being assumed to be equal to 383.0. The degree of dissociation, β', of the hydrochloride of the glycocoll is put equal to the degree of dissociation of ammonium chloride solutions of the same equivalent concentration, $\mu_\infty = 130.1$. The concentration of the glycocoll is throughout $a = 0.4$ normal.

The calculations after Formula (1) are set forth in Table 14, those after Formula (2, 3) in Table 15. In order to make distinct even small differences between the results of the two methods of calculation, we have reckoned with a greater number of decimals than is really warranted by the exactness with which the used constants are known.

TABLE 14.

Calculation after Formula (1) of the Hydrogen-ion Concentration, h, in mixtures of Glycocoll and Hydrochloric Acid; $a = 0.4$.

No. of exp-	Concentration of hydrochloric acid added s $s \cdot 10^3$	α	β'	Hydrogen-ion concentration, h, calculated $h \cdot 10^6$	$\div$ log. h	$\div$ log. s
1	0.0010	1.000	1.000	0.0108	7.967	6.000
2	0.0100	1.000	0.999	0.1078	6.967	5.000
3	0.0336	0.999	0.998	0.3619	6.441	4.474
4	0.0638	0.998	0.996	0.6850	6.164	4.195
5	0.1000	0.997	0.993	1.0704	5.970	4.000
6	0.1733	0.995	0.991	1.8516	5.732	3.761
7	0.3211	0.991	0.988	3.426	5.465	3.493
8	0.6418	0.987	0.983	6.810	5.167	3.193
9	1.0000	0.985	0.978	10.575	4.976	3.000
10	1.8442	0.982	0.971	19.394	4.712	2.734
11	4.5842	0.975	0.957	47.861	4.320	2.339
12	10.0000	0.966	0.939	103.89	3.983	2.000

[1]) From measurements made by S. Palitzsch and K. G. Dernby (see Comptes-rendus du. Laboratoire de Carlsberg, **11**, 268 (1916)).

TABLE 15.

Calculation after Formulas (2, 3) of the added Hydrochloric Acid Concentration in mixtures of Glycocoll and Hydrochloric Acid when the hydrogen-ion concentration, h, is given; $a = 0.4$.

No. of exp.	Hydrogen-ion concentration h $h \cdot 10^6$	a	β'	Acid concentration, s, calculated $s \cdot 10^3$	$\div \log. h$ (p_H)	$\div \log. s$
13	0.696	1.000	1.000	0	6.157	∞
14	0.750	1.000	0.999	0.009822	6.125	5.008
15	0.900	0.999	0.998	0.03371	6.046	4.472
16	1.120	0.998	0.996	0.06405	5.951	4.193
17	3.447	0.991	0.988	0.3099	5.463	3.509
18	10.60	0.985	0.978	0.9975	4.975	3.001
19	47.92	0.975	0.957	4.586	4.319	2.339
20	103.80	0.966	0.939	9.990	3.984	2.000

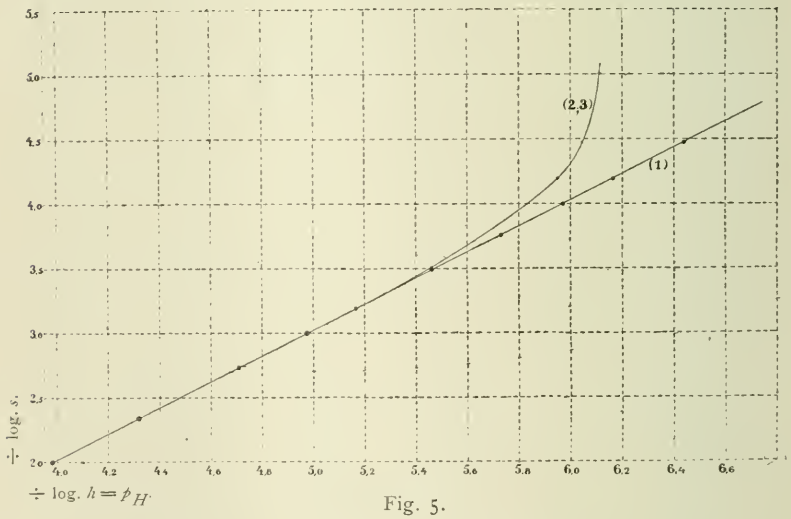

Fig. 5.

It appears from the tables, and with especial clearness from the graphical representation of the results of the calculations in Fig. 5, that either formula practically gives the same result, if the addition of acid is so great that the hydrogen-ion concentration

is equal or superior to about $4 \cdot 10^{-6}$ (p_H = abt. 5.4). At lower hydrogen-ion concentrations the use of Formula (1) gives rise to errors, and the smaller the addition of acid, and consequently the hydrogen-ion concentration, the greater will such errors be.

It is possible to form a fairly correct estimate of the extent of the error at different hydrogen-ion concentrations, if we consider that the main error of Formula (1) is a consequence of the circumstance that we have not taken into account the dissociation of the ampholyte as an acid, to which dissociation the following state of equilibrium applies (see p. 95):

$$h \cdot \varepsilon = k_a \cdot u .$$

If the concentration of added acid is 0, the hydrogen-ion concentration, which we will designate h_o, will be well-nigh completely determined by this dissociation equation, and h_o will be equal to ε_o. The error expressed in % of the hydrogen-ion concentration will of course in this case be 100. If a little dose of some acid is added, the hydrogen-ion concentration will be increased and the dissociation of the ampholyte as acid will be pushed back; whilst previously we had

$$h_o \cdot \varepsilon_o = h_o \cdot h_o = k_a \cdot u_o ,$$

we now get

$$h_1 \cdot \varepsilon_1 = k_a \cdot u_1 .$$

In this calculation the hydrogen-ion concentration, which originates in the dissociation of the ampholyte as an acid, may be supposed equal to ε_1 and the question will then be: How great is ε_1 expressed in % of h_1?

We put

$$h_1 = q \cdot h_o ,$$

and then we get

$$\varepsilon_1 = \frac{k_a \cdot u_1}{h_1} = \frac{k_a \cdot u_1}{h_o \cdot q} .$$

If we now put $u_1 = u_o$ — which is permissible provided that the dissociated part of the ampholyte is very small as compared with the undissociated part —, the error expressed in % of the hydrogen-ion concentration will be

$$\frac{100 \cdot \varepsilon_1}{h_1} = \frac{100 \cdot k_a \cdot u_1}{q \cdot h_o \cdot q \cdot h_o} = \frac{100 \cdot k_a \cdot u_o}{q \cdot h_o \cdot q \, h_o} = \frac{100}{q^2}$$

since $h_o \cdot h_o = k_a \cdot u_o$.

From these considerations it appears that, *while the error made in using Formula (1) in the case of a pure aqueous solution of an ampholyte will amount to 100 % of the hydrogen-ion concentration of the solution, the error in an acid solution of an ampholyte with a q times greater hydrogen-ion concentration will only come to* $\frac{100}{q^2}$ *% of the hydrogen-ion concentration of the latter solution.*

In order to elucidate this question more thoroughly, we have in Table 16 set forth the results calculated by Formulas (2, 3) (Table 15), together with the corresponding values resulting from Formula (1).

TABLE 16.

Magnitude of the Error committed by the use of Formula (1) for the calculation of the Hydrogen-ion concentration in very weak hydrochloric solutions of Glycocoll.

Concentration of acid s	Hydrogen-ion concentration of the solution		Error, ε, committed by the use of Formula (1) ($\varepsilon = h - h'$)	$q = \frac{h}{h_o}$ ($h_o = 0.696 \cdot 10^{-6}$)	ε in % of h	$\varepsilon = \frac{100}{q^2}$
	by Formulas (2, 3)	by Formula (1)				
$s \cdot 10^3$	$h \cdot 10^6$	$h' \cdot 10^6$	$\varepsilon \cdot 10^6$			
0	0.696	0	0.696	1.000	100.00	100.00
0.009822	0.750	0.106	0.644	1.078	85.87	86.05
0.03371	0.900	0.363	0.537	1.293	59.67	59.81
0.06405	1.120	0.687	0.433	1.609	38.66	38.63
0.3099	3.447	3.311	0.136	4.953	3.95	4.08
0.9975	10.60	10.56	0.04	15.23	0.38	0.43
4.586	47.92	47.92	0	68.85	0	0.02
9.990	103.80	103.80	0	149.14	0	0.00

The two first columns in Table 16 are directly copied from Table 15. The figures in the third column state the hydrogen-ion concentrations, calculated after Formula (1), at the acid concentrations given in the first column, and they are found graphically by means of Fig. 5, drawn to a sufficiently large scale. In this connection it may furthermore be noted that Table 14 contains calculations of the hydrogen-ion concentration in solutions of much the same acid concentrations, so it has been possible to effect the interpolation very exactly. The other columns

of the Table 16 do not require any further explanation; we shall only advert to the perfect accordance between the difference found by the two methods of calculation and the error calculated after the expression $\frac{100}{q^2}$ (the last columns in Table 16).

b. The Hydrogen-ion Concentration in salt-containing solutions of Ampholytes.

We shall first consider the case where the salt present in the ampholyte solution is one formed by a strong acid and a strong base, and provisionally disregarding the dissociation of the ampholyte into hydrogen- and anions and into hydroxyl- and kations, respectively.

We use the same designations as before, while the concentration of the salt, MAc, is termed c (degree of dissociation β).

If now the concentration of the base combined with the ampholyte is put equal to x, and if s and y, as before, indicate the concentrations of added and of free acid, respectively, the concentration of the fixed acid will be $s + x \div y$, and the concentration of the salt: $c' = c \div x$. If β'' indicates the degree of dissociation of the salt of the ampholyte with the base, MOH, the concentrations of the different compounds and ions in the solution will be the following:

	[RHOH]	[RH$^+$]	[ROH$\div$]	[RHAc]	[ROHM]	[HAc]
Conc.	u	$(s+x\div y)\beta'$	$x\cdot\beta''$	$(s+x\div y)(1\div\beta')$	$x(1\div\beta'')$	$y(1\div\alpha)$

	[Ac$\div$]	[M$^+$]	[MAc]	[H$^+$]	[OH$\div$]
Conc.	$y\alpha+(s+x\div y)\beta'+c'\beta$	$x\beta''+c'\beta$	$c'(1\div\beta)$	h	$\frac{k_w}{h}$.

These quantities are connected by the following five equations:

1) The dissociation of the ampholyte as an acid:

$$[H^+]\,[ROH\div] = k_a\,[RHOH]$$
$$h\cdot x\cdot\beta'' = k_a\cdot u$$

2) The dissociation of the ampholyte as a base:

$$[OH\div]\,[RH^+] = k_b\,[RHOH]$$
$$\frac{k_w}{h}(s + x \div y)\beta' = k_b\cdot u$$

3) The dissociation of the acid:

$$h = y\cdot a$$

4) $a = u + (s + x \div y)\,\beta' + x\,\beta'' + (s + x \div y)\,(1 \div \beta') + x\,(1 \div \beta'')$

$a = u + s + 2x \div y$

5) $c = c' + x.$

If k_a, k_b, k_w, a, β', β'', a, c and s are known, the five unknown quantities, x, y, u, h and c', may be found from the above five equations. If x, u and h are eliminated from the first four equations, we shall get the following equation:

$$y^3 + y^2\left(a \div s + \frac{k_w \cdot \beta'}{k_b \cdot a}\right) \div \frac{k_w \cdot \beta'}{k_b \cdot a}\left(y \cdot s + \frac{k_a}{a \cdot \beta''}(a + s \div y)\right) = 0.$$

If s and consequently y are not very small, we may in the parenthesis in the last term of the equation disregard the quantity $\frac{k}{a \cdot \beta''}(a + s \div y)$, which is minimal as compared with the product $y \cdot s$. The equation is thus reduced to:

$$y^2 + y\left(a \div s + \frac{k_w \cdot \beta'}{k_b \cdot a}\right) \div s \cdot \frac{k_w \cdot \beta'}{k_b \cdot a} = 0;$$

$$y = \div \frac{a \div s + \frac{k_w \cdot \beta'}{k_b \cdot a}}{2} \pm \sqrt{\frac{\left(a \div s + \frac{k_w \cdot \beta'}{k_b \cdot a}\right)^2}{4} + s \cdot \frac{k_w \cdot \beta'}{k_b \cdot a}},$$

and this expression is identical to Formula (1) (see p. 94). The amount of free acid, and hence the hydrogen-ion concentration, in solutions of an ampholyte and not too small amounts of acid may — in presence of salts of strong bases and strong acids — be calculated in quite the same manner as in salt-free solutions. The influence of the salt only appears by the fact that the values of a and β', of course, vary with the concentration of the salt.

If the salt contained in the solution is derived from a weak acid or weak base, or from both, the state of things will be somewhat more complicated. We will here consider the case where the salt is derived from a strong acid and weak base, as, for instance, in solutions containing ammonium sulphate, which are of special interest to us. We may here make the same reflections as above; yet we are obliged to reckon with the circumstance that the solution, besides free acid (conc. y), will also contain free base, MOH, originating in the hydrolysis of the salt. We will put the concentration of the base equal to z. If we use the same designations as above, the concentration of

the fixed base will be $x \div z$, and the concentration of fixed acid $s + x \div y$; the concentration of fixed "surplus" acid will thus be $s + x \div y \div (x \div z) = s \div y + z$.

If we now, as above, leave out of consideration the dissociation of the ampholyte into hydrogen- and anions or into hydroxyl- and kations, the different compounds and ions in the solution will have the following concentrations:

	[RHOH]	[RH+]	[ROH÷]	[RHAc]
Conc.	u	$(s + x \div y)\beta'$	$(x \div z)\beta''$	$(s + x \div y)(1 \div \beta')$

	[ROHM]	[HAc]
Conc.	$(x \div z)(1 \div \beta'')$	$y(1 \div \alpha)$

	[Ac÷]	[MOH]	[M+]	[MAc]
Conc.	$y\alpha + (s + x \div y)\beta' + c'\beta$	z	$(x \div z)\beta'' + c'\beta$	$c'(1 \div \beta$

	[H+]	[OH÷]
Conc.	h	$\frac{k_w}{h}$

These quantities are connected by six equations, five of which correspond exactly with those stated above (p. 101), and they will here get the following form:

1) $h \cdot (x \div z)\beta'' = k_a \cdot u$

2) $\frac{k_w}{h}(s + x \div y)\beta' = k_b \cdot u$

3) $h = y \cdot \alpha$

4) $a = u + s + 2x \div y \div z$

5) $c = c' + x$.

To these are in the present case to be added:

6) The dissociation equation of the weak base:

$$[OH^{\div}][M^+] = k_m[MOH]$$

$$\frac{k_w}{h}((x \div z)\beta'' + c'\beta) = k_m \cdot z.$$

If k_a, k_b, k_m, k_w, α, β, β', β'', a, c and s are known, the six unknown quantities x, y, z, u, h and c' are determined by the above six equations. By elimination of x, y, z, u and c', we get the following expression:

$$s = \frac{a\left(\frac{k_b \cdot h}{k_w \cdot \beta'} \div \frac{k_a(k_m \cdot h + k_w \cdot \beta'')}{h\beta''(k_m \cdot h + k_w \cdot \beta)}\right)}{1 + \frac{k_a}{h \cdot \beta''} + \frac{h \cdot k_b}{\beta' \cdot k_w}} + \frac{h}{\alpha} \div \frac{c \cdot \beta \cdot k_w}{k_m \cdot h + k_w \cdot \beta} \quad (4).$$

It is difficult to make use of this equation if we consider h as unknown, while it is possible by comparatively simple calculations to find s for a given value of h. Therefore we do not calculate the hydrogen-ion concentration for a certain concentration of added acid, but on the contrary we calculate what proportion of acid must be added in order to give the solution a certain hydrogen-ion concentration. If s and h are known, it is easy to calculate the other unknown quantities.

In Section *a* we accomplished exactly the calculation of the hydrogen-ion concentration in salt-free, acid-containing solutions of ampholytes, while also making allowance for the dissociation of the ampholyte into hydrogen- and anions or into hydroxyl- and kations. It will be too complicated to carry through such a calculation also in the case of salt-containing ampholyte solutions; but we hardly commit any great error if we consider the state of things in salt-containing and salt-free solutions as analogous, and apply the above considerations to salt-containing ampholyte solutions as well.

Only in one case we will try to carry through our calculations on as wide a basis as possible: — we will calculate the common expression of the amount of "surplus" acid or base combined with one equivalent ampholyte, also taking into consideration the dissociation of the ampholyte into hydrogen- and anions or into hydroxyl- and kations, respectively Then we will try from this expression to draw conclusions as to the influence exerted by the salt concentration upon the capacity of the ampholyte to combine with acids or bases.

We use the same designations as above, so that δ signifies the concentration of the ampholyte kations formed by the dissociation of the ampholyte as a base, and ε the concentration of the ampholyte anions formed by the dissociation of the ampholyte as an acid. We have then the following concentrations in the solution:

	[RHOH]	[RH$^+$]	[ROH$^\div$]	[RHAc]
Conc.	u	$(s + x \div y)\beta' + \delta$	$(x \div z)\beta'' + \varepsilon$	$(s + x \div y)(1 \div \beta')$

	[ROHM][1]
Conc.	$(x \div z)(1 \div \beta'')$

[1]) We do not take into consideration either the ions with two opposite charges, R+÷, or the ions RM+ and RAc÷.

Among the above equations (p. 103) we use 1), 2) and 4), which in this case assume the following forms:

1) $h\,((x \div z)\,\beta'' + \varepsilon) = k_a \cdot u$

2) $\frac{k_w}{h}\,((s + x \div y)\,\beta' + \delta) = k_b \cdot u$

4) $a = u + s \div x \div y + \delta + x \div z + \varepsilon.$

The concentration of fixed surplus acid may, as before, be expressed by $s \div y + z$; if this expression becomes negative, this means that the ampholyte is combined with surplus base. The concentration of the acid combined with one equivalent ampholyte: $\frac{s \div y + z}{a}$ may be calculated by means of the above three equations and expressed as follows:

$$\frac{s \div y + z}{a} = \frac{\left(\frac{k_b \cdot h}{k_w \cdot \beta'} \div \frac{k_a}{h \cdot \beta''}\right)\left(1 + \frac{1}{a}\left(\frac{\delta\,(1 \div \beta')}{\beta'} + \frac{\varepsilon\,(1 \div \beta'')}{\beta''}\right)\right)}{1 + \frac{k_b \cdot h}{k_w \cdot \beta'} + \frac{k_a}{h \cdot \beta''}} + \frac{1}{a}\left(\frac{\varepsilon}{\beta''} \div \frac{\delta}{\beta'}\right)$$

This expression may also be written:

$$\frac{s \div y + z}{a} = \frac{\left(\frac{k_b}{[OH \div]} \div \frac{k_a}{[H^+]} \cdot \frac{\beta'}{\beta''}\right)\left(1 + \frac{1}{a}\left(\frac{\delta\,(1 \div \beta')}{\beta'} + \frac{\varepsilon\,(1 \div \beta'')}{\beta''}\right)\right)}{\beta' + \frac{k_b}{[OH \div]} + \frac{k_a}{[H^+]} \cdot \frac{\beta'}{\beta''}} + \frac{1}{a}\left(\frac{\varepsilon}{\beta''} \div \frac{\delta}{\beta'}\right) \quad (5)$$

If now we consider an ampholyte the aqueous solution of which shows an acid reaction ($k_a > k_b$), ε will be greater than δ; but either of these quantities will always be very small, and in most cases quite minimal, as compared with the other quantities in Equation (5). If we neglect all the terms which contain ε and δ, Equation (5) will get the following simpler form:

$$\frac{s \div y + z}{a} = \frac{\frac{k_b}{[OH \div]} \div \frac{k_a}{[H^+]} \cdot \frac{\beta'}{\beta''}}{\beta' + \frac{k_b}{[OH \div]} + \frac{k_a}{[H^+]} \cdot \frac{\beta'}{\beta''}} \quad (6)$$

As now β' diminishes with increasing salt concentration, while $\frac{\beta'}{\beta''}$ undergoes but a slight modification, it follows that the whole expression (6) will grow greater with increasing salt concentration.

If further we might put $\frac{\beta'}{\beta''} = 1$, the numerator of expression (6) would be equal to 0 at the isoelectric reaction, at which hydrogen-ion concentration we have $\frac{k_b}{[OH^-]} = \frac{k_a}{[H^+]}$ [1]). As now $\frac{\beta'}{\beta''}$ will usually differ a little from 1, the hydrogen-ion concentration at which the numerator of the fraction becomes 0 will not coincide exactly with the isoelectric point of the ampholyte; but for brevity's sake we may say that the numerator of the fraction becomes 0 at the isoelectric reaction.

Our Equation (6) will then express that the concentration of the surplus acid combined with one equivalent ampholyte

1) is independent of the concentration of the ampholyte, because the quantity a does not enter into the right side of Equation (6);
2) increases with rising salt concentration (decreasing β');
3) is positive if the hydrogen-ion concentration is greater than that corresponding to the isoelectric reaction, — and
4) is negative, that is: the ampholyte is combined with surplus base, if the hydrogen-ion concentration is inferior to that corresponding to isoelectric reaction.

Further, Equation (6) says that at isoelectric reaction the ampholyte has neither combined with surplus acid nor with surplus base; but just at the isoelectric reaction and at hydrogen-concentrations in the neighbourhood of this reaction the simple Equation (6) is not applicable, since in this case we are not justified in neglecting ε and δ, but are obliged to resort to the more complicated Equation (5).

From Equation (5) is seen that at the isoelectric reaction (that is, in our case, if $\frac{k_b}{[OH^-]} = \frac{k_a}{[H^-]} \cdot \frac{\beta'}{\beta''}$)

$$\frac{s - y + z}{a} = \frac{1}{a}\left(\frac{\varepsilon}{\beta''} - \frac{\delta}{\beta'}\right).$$

As ε is greater than δ, while β' and β'' are about equal to each other, the ampholyte will consequently at isoelectric reaction combine with surplus acid; and further it is seen that the concentration of the acid combined with one equivalent ampholyte —

[1]) That this relation will also apply to salt-containing solutions, is seen from the above equations, since 1) and 2) (p. 105) will give $\frac{k_b \cdot u \cdot h}{k_w} = \frac{k_a \cdot u}{h}$ if $[RH^+] = [ROH^-]$.

in contradistinction to the result obtained from Equation (6) — is not independent of the ampholyte concentration, but is the greater as the ampholyte concentration grows less, since ε certainly decreases with a, but not so fast.

It would of course be possible from Equation (5) to find an expression for the conditions in which the ampholyte will neither combine with surplus acid nor base; but the expression would be too complicated to permit us to draw simple conclusions. The only fact which a more thorough study of Equation (5) is able to prove is, that the nearer the hydrogen-ion concentration is to the isoelectric reaction, the greater will be the influence of the ampholyte concentration. It is only at hydrogen-ion concentrations which are somewhat greater or smaller than that corresponding with isoelectric reaction that the concentration of surplus acid or base combined with one equivalent ampholyte is independent of the ampholyte concentration.

We arrive at a similar expression as Equation (5), but a little simpler, if we carry out an analogous calculation for acid-containing but salt-free ampholyte solutions, and we may from this expression draw similar conclusions as from the Equations (5) and (6).

c. Experimental Part; Measurements of Hydrogen-ion Concentrations in acid-containing, salt-free as well as salt-containing, solutions of Ampholytes of simple composition.

(Glycocoll and Glycylglycine.)

In order to ascertain the exactness with which it is possible to execute the measuring of the hydrogen-ion concentration in acid solutions of ampholytes, and from this to calculate the capacity of the ampholyte to combine with acids, we shall in this section set forth a series of values (found by measurings of hydrogen-ion concentrations) of the capacity of glycocoll and glycylglycine to combine with acids and the corresponding quantities calculated by means of the formulas given in Sections *a* and *b*.

The results of such calculations by means of Formula (1) (see p. 94) are set forth in Table 17 in the case of salt-free mixtures of glycocoll and hydrochloric acid, in Table 19 in the case of saltfree mixtures of glycylglycine and hydrochloric acid,

and in Table 18 in the case of mixtures of glycocoll and hydrochloric acid containing sodium chloride. In these calculations we used for glycocoll the dissociation constants mentioned above (p. 97), for glycylglycine: $k_a = 3.2 \cdot 10^{\div 9}$ and $k_b = 9.3 \cdot 10^{\div 12}$[1]); the degree of dissociation of the hydrochloride of the glycylglycine was put equal to the degree of dissociation of ammonium chloride (see p. 97), just as in the case of glycocoll. Each of the Tables 17, 18 and 19 further contains a series of values of the hydrogen-ion concentration found by hydrogen-ion measurements in such mixtures of ampholyte and hydrochloric acid, and finally the capacity (calculated therefrom) of the ampholyte to combine with hydrochloric acid, expressed as the number, b, of milligram-equivalents of hydrochloric acid combined with one milligram-equivalent of ampholyte-nitrogen.

It appears from the tables — the details of which require no further explanation —, that the accordance between the calculated and the found quantities is, on the whole, remarkably satisfactory; only at very high hydrogen-ion concentrations does a slight discrepancy appear between the found and the calculated values.

The very satisfactory accordance between calculation and experiment appears with especial clearness in the graphical representation in the Figures 6, 7 and 8, where the curves are drawn on the basis of the calculated values, while the values found by the measurements are plotted on the curves after these had been drawn up. A more detailed account of these curves will be given later on (p. 115).

In Table 20 we give the calculation, after Formula (4) (p. 103) of the sulphuric acid concentration which is necessary to give mixtures of glycocoll and ammonium sulphate a certain hydrogen-ion concentration, while in Table 21 we set forth the results of some hydrogen-ion measurements executed by Miss J. Hempel in such ammonium-sulphate-containing solutions of glycocoll with known contents of surplus sulphuric acid, s. In calculating Table 20 we used the values of α and β stated in Table 7 (see p. 80) for the given ammonium sulphate concentration, c. Concerning β' (the degree of dissociation of the salt of glycocoll with the acid) and β'' (the degree of dissociation of the ammonium salt of the

[1]) After measurements by K. G. Dernby (Comptes-rendus du Laboratoire de Carlsberg, **11**, 269 (1916)).

TABLE 17.

The Hydrogen-ion concentration of mixtures of Hydrochloric Acid and Glycocoll and the Capacity of the Glycocoll to combine with Hydrochloric Acid calculated from the measurements.

Concentration of glycocoll, a, in all mixtures: $a = 400 \cdot 10 \div 3$.

No. of Exp.	Concentration of hydrochloric acid added s $s \cdot 10^3$	$\div$ log. s	α	β'	Calculated: Concentration of free hydrochloric acid y $y \cdot 10^3$	Calculated: Hydrogen-ion concentration, $h = \alpha \cdot y$ $h \cdot 10^3$	Calculated: p_H	Calculated: Number of milligr. equiv. hydrochloric acid *combined* with one milligr. equiv. glycocoll nitrogen, $b = \frac{s \div y}{a}$ $b \cdot 10^3$	Calculated: $\div$ log. b	Found by hydrogen-ion measurement: Hydrogen-ion-concentration, h $h \cdot 10^3$	Found by hydrogen-ion measurement: p_H	Found by hydrogen-ion measurement: Concentration of free hydrochloric acid, $y = \frac{h}{\alpha}$ $y \cdot 10^3$	Found by hydrogen-ion measurement: Number of milligr. equiv. hydrochloric acid *combined* with one milligr. equiv. glycocoll nitrogen, $b = \frac{s \div y}{a}$ $b \cdot 10^3$	Found by hydrogen-ion measurement: $\div$ log. b
1	1	3.000	0.985	0.98	0.01074	0.01057	4.976	2.47	2.607	—	—	—	—	—
2	10	2.000	0.966	0.94	0.1076	0.1039	3.983	24.7	1.607	0.1016	3.993	0.1052	24.7	1.607
3	20	1.699	0.958	0.92	0.2180	0.2088	3.680	49.5	1.305	0.2109	3.676	0.2201	49.5	1.305
4	30	1.523	0.950	0.91	0.3350	0.3183	3.497	74.2	1.130	0.3214	3.493	0.3383	74.2	1.130
5	40	1.398	0.945	0.90	0.4550	0.4300	3.367	98.9	1.005	0.4385	3.358	0.4640	98.8	1.005
6	50	1.301	0.940	0.89	0.5850	0.5499	3.260	123.5	0.908	0.5534	3.257	0.5887	123.5	0.908
7	60	1.222	0.935	0.88	0.7135	0.6671	3.176	148.2	0.829	0.6865	3.164	0.7342	148.2	0.829
8	80	1.097	0.926	0.86	0.9970	0.9232	3.035	197.5	0.704	0.9840	3.007	1.0626	197.3	0.705
9	100	1.000	0.917	0.85	1.325	1.215	2.915	246.7	0.608	1.306	2.884	1.424	246.4	0.608
10	200	0.699	0.893	0.82	3.850	3.438	2.464	490.4	0.309	3.954	2.403	4.428	488.9	0.311
11	300	0.523	0.877	0.80	10.435	9.151	2.039	723.9	0.140	10.91	1.962	12.44	718.9	0.143
12	400	0.398	0.863	0.79	38.005	32.80	1.484	905.0	0.043	39.17	1.407	45.39	886.5	0.052
13	500	0.301	0.854	0.78	113.545	96.97	1.013	966.1	0.015	106.42	0.973	124.61	938.5	0.028

TABLE 18. **The Hydrogen-ion concentration of mixtures of Hydrochloric Acid, Glycocoll and Sodium Chloride, and the Capacity of Glycocoll to combine with Hydrochloric Acid calculated from the measurements.**

The whole concentration of hydrochloric acid and sodium chloride was in all the mixtures $100 \cdot 10^{\div 3}$; therefore $\alpha = 0.917$ and $\beta' = 0.85$ were used in all the calculations.

No. of Exp.	Concentration of glycocoll, a	Concentration of hydrochloric acid added s	Calculated: Concentration of free hydrochloric acid, y	Calculated: Hydrogen-ion-concentration, $h = \alpha \cdot y$	Calculated: p_H	Calculated: Number of milligr.-equiv. hydrochloric acid *combined* with one milligr. equiv. glycocoll nitrogen, $b = \frac{s \div y}{a}$	Calculated: $\div$ log. b	Found by hydrogen-ion measurement.[1]: Hydrogen ion concentration, h	Found: p_H	Found: Concentration of free hydrochloric acid, $y = \frac{h}{\alpha}$	Found: Number of milligr.-equiv. hydrochloric acid *combined* with one milligr. equiv. glycocoll nitrogen, $b = \frac{s \div y}{a}$	Found: $\div$ log. b
	$a \cdot 10^3$	$s \cdot 10^3$	$y \cdot 10^3$	$h \cdot 10^3$		$b \cdot 10^3$		$h \cdot 10^3$		$y \cdot 10^3$	$b \cdot 10^3$	
14	99	1	0.0397	0.0364	4.439	9.7	2.013	0.0388	4.411	0.0423	9.7	2.013
15	97.5	2.5	0.1018	0.0934	4.030	24.6	1.609	0.1021	3.991	0.1113	24.5	1.611
16	95	5	0.2143	0.1965	3.707	50.4	1.298	0.2094	3.679	0.2284	50.2	1.299
17	90	10	0.4780	0.4383	3.358	105.8	0.976	0.4560	3.341	0.4973	105.6	0.976
18	80	20	1.238	1.135	2.945	234.5	0.630	1.197	2.922	1.305	233.7	0.631
19	70	30	2.599	2.383	2.623	391.4	0.407	2.472	2.607	2.696	390.1	0.409
20	60	40	5.475	5.021	2.299	575.4	0.240	5.260	2.279	5.736	571.1	0.243
21	50	50	12.34	11.32	1.946	753.2	0.123	11.70	1.932	12.76	744.8	0.128
22	40	60	25.48	23.37	1.631	863.0	0.064	22.65	1.645	24.70	882.5	0.054
23	30	70	42.60	39.06	1.408	913.3	0.039	38.11	1.419	41.56	948.0	0.023
24	20	80	61.24	56.16	1.251	938.0	0.028	56.11	1.251	61.19	940.5	0.027
25	10	90	80.48	73.80	1.132	952.0	0.021	71.45	1.146	77.92	(1208)	$\div$ 0.082
26	0	100	100.00	91.70	1.038	—	—	91.62	1.038	99.91	—	—

[1]) Comptes rendus du Laboratoire de Carlsberg, **8**, 41 (1909).

TABLE 19. **The Hydrogen-ion concentration in mixtures of Hydrochloric Acid and Glycylglycine and the Capacity of Glycylglycine to combine with Hydrochloric Acid calculated from the measurements.**

In all mixtures the mol. concentration of glycylglycine is $a = 200 \cdot 10 \div 3$; the concentration of glycylglycine nitrogen is therefore $2a = 400 \cdot 10 \div 3$.

No. of exp.	Concentration of hydrochloric acid added, s	$\div \log s$	α	β'	Calculated: Concentration of free hydrochloric acid, y	Calculated: Hydrogen ion concentration, $h = \alpha \cdot y$	P_H	Calculated: Number of milligr.-equiv. hydrochloric acid, *combined* — with one milligr.-equiv. glycylglycine, $B = \frac{s \div y}{a}$	Calculated: Number of milligr.-equiv. hydrochloric acid, *combined* — with one milligr.-equiv. glycylglycine-nitrogen, $b = \frac{B}{2}$	$\div \log b$	Found by hydrogen-ion measurement [1]: Hydrogen-ion-concentration, h	Found: P_H	Found: Concentration of free hydrochloric acid $y = \frac{h}{\alpha}$	Found: Number of milligr.-equiv. hydrochloric acid, *combined* — with one milligr. equiv. glycylglycine, $B = \frac{s \div y}{a}$	Found: with one milligr.-equiv. glycylglycine nitrogen, $b = \frac{B}{2}$	$\div \log b$
	$s \cdot 10^3$				$y \cdot 10^3$	$h \cdot 10^3$		$B \cdot 10^3$	$b \cdot 10^3$		$h \cdot 10^3$		$y \cdot 10^3$	$B \cdot 10^3$	$b \cdot 10^3$	
27	1	3.000	0.985	0.98	0.0039	0.0038	5.420	4.98	2.49	2.604	—	—	—	—	—	—
28	10	2.000	0.966	0.94	0.0395	0.0382	4.418	49.8	24.9	1.604	—	—	—	—	—	—
29	20	1.699	0.958	0.92	0.0822	0.0787	4.104	99.6	49.8	1.303	0.0760	4.119	0.0793	99.6	49.8	1.303
30	40	1.398	0.945	0.90	0.1832	0.1731	3.762	199.0	99.5	1.002	0.1726	3.763	0.1826	199.0	99.5	1.002
31	60	1.222	0.935	0.88	0.3097	0.2896	3.538	298.4	149.2	0.826	0.2924	3.534	0.3127	298.4	149.2	0.826
32	80	1.097	0.926	0.86	0.4746	0.4395	3.357	397.6	198.8	0.702	0.4645	3.333	0.5016	397.4	198.7	0.702
33	100	1.000	0.917	0.85	0.7069	0.6482	3.188	496.4	248.2	0.605	0.7047	3.152	0.7685	496.2	248.1	0.605
34	130	0.886	0.909	0.84	1.291	1.174	2.930	643.6	321.8	0.492	—	—	—	—	—	—
35	160	0.796	0.901	0.83	2.632	2.371	2.625	786.8	393.4	0.405	—	—	—	—	—	—
36	200	0.699	0.893	0.82	11.575	10.336	1.986	942.1	471.1	0.327	—	—	—	—	—	—

[1]) K. G. Dernby: Comptes-rendus du Laboratoire de Carlsberg **11**, 269 (1916).

glycocoll), no measurements for calculation of these quantities have ever been published, as far as we know. By the use of Formula (4) it appeared, however, that many of the quantities in Formula (4) are of little or no consequence, provided that h is chosen fairly high ($20 \cdot 10^{\div 5}$ or more). The formula is thus reduced to the simple expression:

$$s = \frac{a \cdot \frac{k_b}{k_w} \cdot h}{\beta' + \frac{k_b}{k_w} \cdot h} + \frac{h}{a} \qquad (7).$$

Formula (7) permits a calculation of β', if s is known and h is measured, and we have therefore used a few of the experiments stated in Table 21 for the calculation of β'.

The quantity β'' is of no material consequence; we have supposed it to be equal to the degree of dissociation of potassium acetate in solutions of the same equivalent concentration as the present equivalent concentration of ammonium sulphate.

The figures in Table 20 are easily to be understood, if we remember Formula (4) and the equation from which this formula was deduced (see p. 103). As was to be expected from the explanation given towards the end of the last section (p. 106), the capacity, b, calculated per milligram-equivalent glycocoll, to combine with acids has proved to be independent of the glycocoll concentration, but dependent on the ammonium sulphate concentration, c, since b at the same hydrogen-ion concentration is greater if $c = 2.8$, than it is if $c = 0.35$. It is only at the lowest hydrogen-ion concentration ($1 \cdot 10^{\div 6}$) that the result is different; but this is probably due to the fact that the hydrogen-ion concentration is here so low that it is necessary to make allowance for the quantities above designated as δ and ε (compare p. 106)[1].

Concerning Table 21 we shall only add that the concentration of the free surplus sulphuric acid, s', was calculated from the hydrogen-ion concentration, h, found by the measurement, making allowance for the hydrolysis of the ammonium sulphate (see Section A p. 76), but without paying regard to the volume

[1]) In the calculation of the values of s, x, y, z, u and b, set forth in Table 20, we have used more decimals than those given in the table; this is the reason why the values of b stated in the last but one column of the table sometimes differ a little from those calculated by means of the values of s, y, z, and a in the table.

Table 20. Calculation of the concentration of added Acid, s, for a given hydrogen-ion concentration, h, in mixtures of glycocoll, sulphuric acid and ammonium sulphate (Formula (4)), and of the Capacity of glycocoll to combine with sulphuric acid.

No. of exp.	Concentration of glycocoll, a	Hydrogen-ion concentration, h	P_H	Concentration, s of sulphuric acid calculated of Formula (4)	$\div$ log. s				Number of milligr.-equivalents surplus sulphuric acid combined with one milligr.-equiv. glycocoll, $b = \frac{s \div y + z}{a}$	$\div$ log. b
	$a \cdot 10^3$	$h \cdot 10^6$		$s \cdot 10^3$		$x \cdot 10^3$	$y \cdot 10^3$	$z \cdot 10^3$	$u \cdot 10^3$	$b \cdot 10^3$	
Ammonium sulphate concentration: $2.8 \cdot n/_1$; $\alpha = 0.088$; $\beta = 0.274$; $\beta' = 0.557$; $\beta'' = 0.425$.											
37	400	1	6.000	$\div$ 0.245	—	0.421	0.011	0.316	399.730	0.148	3.830
38	,,	5	5.301	0.793	3.101	0.084	0.057	0.063	399.159	1.999	2.699
39	,,	10	5.000	1.709	2.767	0.042	0.114	0.032	398.353	4.068	2.391
40	,,	30	4.523	5.200	2.284	0.014	0.341	0.011	395.124	12.175	1.915
41	,,	50	4.301	8.617	2.065	0.008	0.568	0.006	391.941	20.137	1.696
42	,,	100	4.000	16.928	1.771	0.004	1.136	0.003	384.203	39.50	1.403
43	,,	1000	3.000	127.903	0.893	0.001	11.364	0.001	283.460	291.35	0.536
44	,,	3000	2.523	254.991	0.593	0.001	34.091	0.001	179.099	552.25	0.258
45	10	1	6.000	$\div$ 0.303	—	0.318	0.011	0.316	9.994	0.148	3.830
46	,,	5	5.301	0.0137	4.863	0.064	0.057	0.063	9.978	1.998	2.699
47	,,	10	5.000	0.123	3.910	0.032	0.114	0.032	9.959	4.068	2.391
48	,,	30	4.523	0.452	3.345	0.011	0.341	0.011	9.878	12.175	1.915
49	,,	50	4.301	0.763	3.117	0.006	0.568	0.006	9.799	20.137	1.696
50	,,	100	4.000	1.528	2.816	0.003	1.136	0.003	9.605	39.50	1.403
Ammonium sulphate concentration: $0.35 \cdot n/_1$; $\alpha = 0.230$; $\beta = 0.395$; $\beta' = 0.688$; $\beta'' = 0.749$.											
51	400	1	6.000	0.0208	4.682	0.117	0.004	0.057	399.806	0.183	3.738
52	,,	5	5.301	0.663	3.178	0.023	0.022	0.011	399.324	1.632	2.787
53	,,	10	5.000	1.359	2.867	0.012	0.043	0.006	398.666	3.303	2.481
54	,,	30	4.523	4.081	2.389	0.004	0.130	0.002	396.043	9.882	2.005
55	,,	50	4.301	6.763	2.170	0.002	0.217	0.001	393.451	16.367	1.786
56	10	1	6.000	$\div$ 0.0507	—	0.058	0.004	0.057	9.996	0.184	3.735
57	,,	5	5.301	0.0267	4.573	0.012	0.022	0.011	9.982	1.631	2.788
58	,,	10	5.000	0.0708	4.150	0.006	0.043	0.006	9.966	3.302	2.481
59	,,	30	4.523	0.227	3.644	0.002	0.130	0.002	9.901	9.881	2.005
60	,,	50	4.301	0.380	3.420	0.001	0.217	0.001	9.836	16.367	1.786

TABLE 21.

Measurements of Hydrogen-ion concentration in mixtures of glycocoll, sulphuric acid and ammonium sulphate, and the Capacity of glycocoll to combine with sulphuric acid calculated from the measurements.

No. of exp.	Concentration of surplus sulphuric acid added, s	$\div$ log. s	Hydrogen-ion concentration, measured		Concentration of surplus free sulphuric acid calculated of the formula $s' = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s}$	Concentration of surplus fixed sulphuric acid $s \div s'$	Number of milligram-equivalents surplus sulphuric acid, combined with one milligram-equivalent glycocoll, $b = \frac{s \div s'}{a}$	$\div$ log. b
	$s \cdot 10^3$		f_H	$h \cdot 10^6$	$s' \cdot 10^3$	$(s \div s') \cdot 10^3$	$b \cdot 10^3$	
Glycocoll conc.: $a = 400 \cdot 10 \div 3$; Ammonium sulphate conc.: $c = 2.80 \cdot n/_1$; $\alpha = 0.088$; $\beta = 0.274$; $(x_{max})^2 = c \frac{k_w \cdot \beta}{k_m \cdot \alpha} = 3587 \cdot 10 \div 12$; $f_s = 11.36$.								
61	0.1001	4.000	5.722	1.90	$\div$ 0.1446	0.2447	0.612	3.213
62	0.2506	3.601	5.624	2.38	$\div$ 0.1056	0.3562	0.891	3.050
63	1.001	3.000	5.222	6.00	0.0155	0.985	2.48	2.606
64	2.004	2.698	4.948	11.27	0.100	1.904	4.76	2.322
65	5.001	2.301	4.546	28.44	0.312	4.689	11.72	1.931
66	10.000	2.000	4.233	58.48	0.659	9.341	23.35	1.632
67	15.023	1.823	4.047	89.74	1.016	14.007	35.02	1.456
68	30.025	1.523	3.743	180.7	2.051	27.974	69.94	1.155
69	100.26	0.999	3.115	767.4	8.72	91.54	228.9	0.640
70	200.64	0.698	2.669	2143	24.34	176.30	440.8	0.356
Glycocoll conc.: $a = 400 \cdot 10 \div 3$; Ammonium sulphate conc.: $0.35 \cdot n/_1$; $\alpha = 0.230$; $\beta = 0.395$; $(x_{max})^2 = c \frac{k_w \cdot \beta}{k_m \cdot \alpha} = 247 \cdot 10 \div 12$; $f_s = 4.35$.								
71	2.003	2.698	4.839	14.49	0.059	1.944	4.86	2.313
72	10.000	2.000	4.123	75.34	0.327	9.673	24.18	1.617

of the glycocoll. The difference between the concentration of the total and the free acid gives the concentration of fixed sulphuric acid, from which the capacity, b, of the glycocoll to combine with acids under the present circumstances can be easily calculated.

For the curves of Fig. 6 we have used p_H· as abscissa and $\div$ log. s as ordinate, and only the mixtures in which the concentration of ampholyte nitrogen is $400 \cdot 10^{\div 3}$ are employed. The

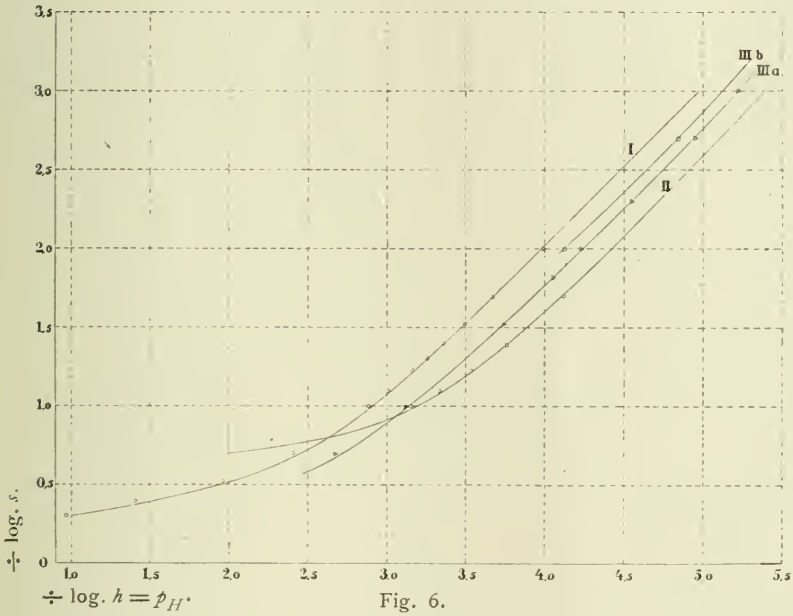

Fig. 6.

curves are drawn after the calculated values, and then the experimental values are plotted.

Curve I corresponds with Table 17 (exp. point: ⊙)
„ II „ „ „ 19 („ „ : ⊙)
„ III a „ „ „ 20 and 21 ($c = 2.8$) (exp. point: △)
„ III b „ „ „ 20 and 21 ($c = 0.35$) („ „ : □).

It appears from Fig. 6, firstly, that the points found by experiments fit perfectly in with the calculated curves.

Secondly it appears that Curve II (that relating to glycylglycine), in the case of low values of s (that is, great values of $\div$ log. s), is situated farthest to the right. The reason is that

glycylglycine is a stronger base, has a greater k_b, than that of glycocoll. The capacity of the glycylglycine mixtures to combine with acids is therefore greater (and the concentration of free acid and the hydrogen-ion concentration consequently smaller, p_H· greater) than that of the glycocoll mixtures. As far as these latter mixtures are concerned, the capacity to combine with acids must, according to what has been explained above (see p. 106), be the greater as the concentration af salt increases, so that the curves must be situated in the following order from the right

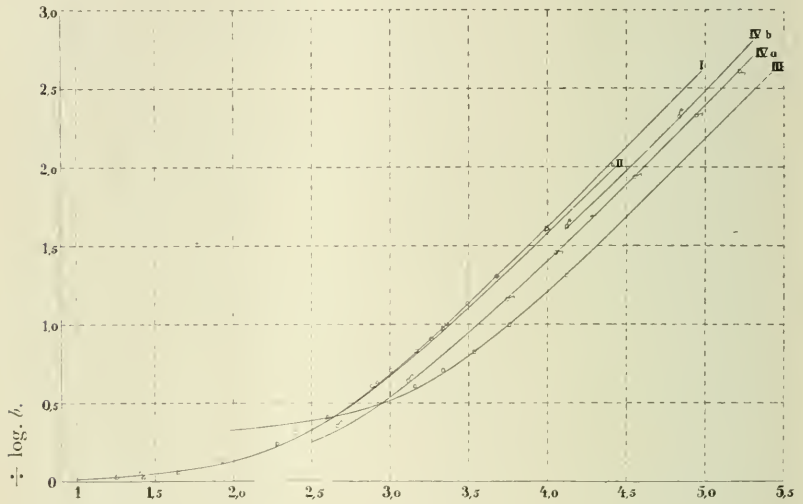

Fig. 7.

to the left: III a (c = 2.8), III b (c = 0.35), I (without addition of salt). Here all the curves run parallel to each other, which means that the capacity to combine with acids and, consequently, the concentration of free acid are modified with increasing s in an analogous way for all the mixtures examined.

At greater values of s (that is, smaller values of ÷ log. s) Curve II and the rest of the curves intersect, because the ampholyte concentration of the glycylglycine mixtures is only one half of what it is in the other mixtures, although the equivalent concentration of nitrogen is the same in all cases.

Quite a similar state of things is met with in Fig. 7, where p_H· is used as abscissa and ÷ log. b as ordinate. As b expresses the capacity to combine with acids per milligram-equivalent of ampholyte nitrogen, we may here use all the measurements, even

those for which the concentration of ampholyte nitrogen is different from $400 \cdot 10^{-3}$.

Curve I corresponds with Table 17 (exp. point: ⊙)
„ II „ „ „ 18 („ „ : △)
„ III „ „ „ 19 („ „ : □)
„ IVa „ „ „ 20 and 21 ($c = 2.8$, exp. point: ♂)
„ IVb „ „ „ 20 and 21 ($c = 0.35$, „ „ : ♂).

Since the capacity to combine with acids, as above explained, must be greater in mixtures of glycylglycine than in those of glycocoll and greater in salt-rich than in salt-poor mixtures, the curves must, if the hydrogen-ion concentration is low — that is, if p_H is high — arrange themselves as follows: III (glycylglycine), IVa ($c = 2.8$), IVb ($c = 0.35$), II ($c = 0.1$), I (without addition of salt).

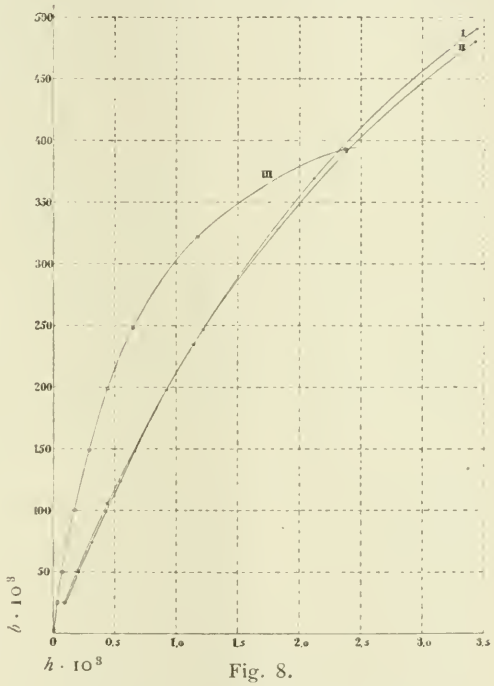

Fig. 8.

The reason why Curve III intersects the other curves at lower values of p_H is explained above; here we shall only draw the attention to the fact that the Curves I and II also intersect, the scale being, however, too small to show this distinctly; it is clearly conspicuous in Fig. 8.

In Fig. 8 h is used as abscissa and b as ordinate, and the scale is chosen so great that we have only been able to plot on the figure that part of the curve-system which corresponds with the lowest hydrogen-ion concentrations. The plotted points were calculated, and the curves drawn accordingly.

Curve I corresponds with Table 17 (calculated point: ○)
„ II „ „ „ 18 („ „ : △)
„ III „ „ „ 19 („ „ : □).

We have mentioned above that b at lower values of h is greater for glycylglycine than for glycocoll. It has further been stated that Curve I and Curve II would intersect, and this appears clearly from Fig. 8. The reason of this fact is that all the mixtures of glycocoll in Table 18 (Curve II) have the same total concentration of chloride, *viz.* 0.1 $^{n}/_{1}$, while the mixtures in Table 17 (Curve I) do not contain sodium chloride, so that the concentration of chloride will increase with increasing addition

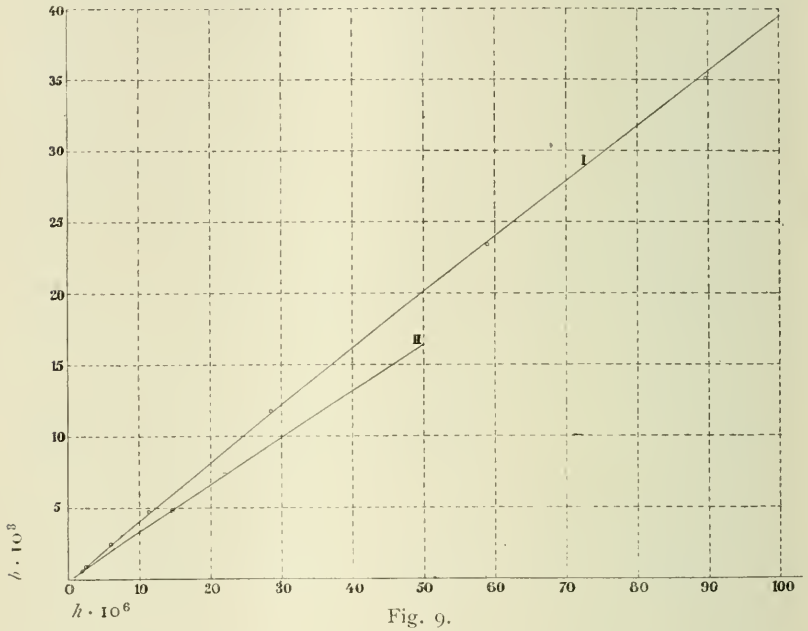

Fig. 9.

of acid. Accordingly, so long as the amount of hydrochloric acid contained in the last-mentioned mixtures is inferior to 0.1 $^{n}/_{1}$, b must be smaller than in the solutions containing sodium chloride, whilst with regard to the mixtures possessing a higher proportion of acid, b must be greater. The two curves must therefore intersect at the point where the chloride concentration is the same, and this will — as shown by Table 18 and Table 17, Experiment No. 9 — be the case when the hydrogen-ion concentration amounts to about $1.2 \cdot 10^{\div 3}$.

Finally, as regards Fig. 9, we have also adopted h as abscissa and b as ordinate. The curves are drawn according to the calculated values, and then the experimental values are plotted;

on account of the chosen scale, however, we have only been able to plot on the figure that part of the system which answer to the lowest concentrations of hydrogen-ions.

Curve I corresponds with Table 20 and 21 ($c = 2.8$; exp. point: ⊙)
„ II „ „ „ 20 „ 21 ($c = 0.35$; „ „ : △).

It is evident from Fig. 9 that the curves which represent the dependence between the capacity of the glycocoll to combine with acids and the hydrogen-ion concentration, are nearly straight lines at the low hydrogen-ion concentrations here in question, and further, that the Curve I ($c = 2.8$), as might have been expected, is situated above Curve II ($c = 0.35$).

C. The Capacity of Egg-albumin to combine with Acids and Bases.

We will now try to apply to solutions of egg-albumin the considerations which in the previous section we advanced with regard to ampholytes of very simple composition. The fact is that solutions of egg-albumin — from a purely chemical point of view — may be supposed to have the character of very complicated amphoteric compounds.

A direct calculation of the capacity of egg-albumin to combine with acids is, of course, rendered out of the question by the circumstance that we do not know the acid and base dissociation constants of the egg-albumin. On the other hand, we will try to find the capacity of egg-albumin to combine with acids by means of measurements of the hydrogen-ion concentration in egg-albumin solutions of known composition in a similar way to that described in the previous section in the case of simple ampholytes, also here leaving out of consideration the dissociation of the egg-albumin into hydrogen- and anions and hydroxyl- and kations, respectively. Such a procedure gives — as pointed out above in the case of simple ampholytes — only correct or approximatively correct results if the hydrogen-ion concentration of the solution is considerably greater or smaller than that corresponding with the isoelectric point of the ampholyte. If this is not so, the method suffers from a source of error, which must not be left out of consideration.

In what follows we are first to describe the method for the execution of the experiments and the results thus obtained, and then mention whether, and if so, how, it will be possible to use the graphical representation of the experimental results for answering the questions made in the introduction to this paper (see p. 68).

a. Determination of the Capacity of Egg-albumin to combine with Acids by means of Measurements of Hydrogen-ion concentrations in ammonium-sulphate-containing solutions of egg-albumin with known contents of surplus sulphuric acid[1]).

The experiments were made with solutions of egg-albumin which had been purified, as described in Paper I (p. 12), by six crystallisations and subsequent dialysis, with repeated additions of small doses of ammonia till the whole amount of sulphuric acid had dialysed away. From such a solution the "stock-solution" was prepared by an exact analysis and subsequent addition of the amount of sulphuric acid equivalent to the slight amount of ammonia found. Besides the stock-solution, whose content of protein nitrogen could be calculated from the analysis, and which contained besides the egg-albumin, only a slight and known amount of ammonium sulphate, but neither surplus sulphuric acid nor ammonia, we used a solution of ammonium sulphate of known composition and with known contents of surplus sulphuric acid or ammonia and, finally, titrated solutions of sulphuric acid or ammonia properly diluted. Weighed amounts of these solutions were mixed and made up with water to 25 cc., and then the hydrogen-ion concentration was measured by Hasselbalch's "Shaking method", and sometimes also while a continued current of hydrogen was passing through the liquid. In each mixture we usually made three measurements with different platinum-hydrogen electrodes, which were measured against two or three different calomel electrodes. In order further to explain the method of calculation by means of the results of the measurings of the capacity to combine with acids, we have in Table 22 reproduced the most important figures resulting from a single series of experiments.

[1]) The amount of acid is here as always — unless the contrary is expressly emphasized — reckoned with the sign in such a way that a negative amount of acid means the equivalent amount of base.

TABLE 22. **Capacity of Egg-albumin to combine with Acids.**

Series of experiments E: $e = 0.370$ n; $f_s = 4.41$; $(x_{max})^2 = 258.5 \times 10^{\div 12}$; $V_a = 96.23$.

No. of exp.	100cc. experimental liquid contained		Hydrogen-ion concentration,	The ammonium sulphate solution surrounding the egg-hydrate in 100 cc. experimental liquid		Amount of sulphuric acid (+) or ammonia (÷) expressed in cc. $^{n}/_{1000}$	
	Protein nitrogen in milligram-equivalents	surplus sulphuric acid (+) or ammonia (÷) in cc. $^{n}/_{1000}$		had the concentration s of surplus sulphuric acid (+) or ammonia (÷)	contained t' cc. $^{n}/_{1000}$ surplus sulphuric acid (+) or ammonia (÷)	corresponding with the whole amount of protein in 100 cc experimental liquid	corresponding with one milligram-equivalent protein nitrogen
	e	t	$h \cdot 10^6$	$s \cdot 10^6$	$t' = V_a \cdot s \cdot 1000$	$t \div t'$	$b = \frac{t \div t'}{e}$
1	42.30	+ 1125.8	80.91	+ 356.1	+ 34.3	+ 1091.5	+ 25.8
2	42.20	+ 1009.9	70.17	+ 308.6	+ 29.7	+ 980.2	+ 23.2
3	42.26	+ 893.4	60.91	+ 267.7	+ 25.8	+ 867.6	+ 20.5
4	42.23	+ 768.2	51.76	+ 227.1	+ 21.9	+ 746.3	+ 17.7
5	42.24	+ 606.3	41.09	+ 179.8	+ 17.3	+ 589.0	+ 13.9
6	42.23	+ 408.7	30.74	+ 133 7	+ 12.9	+ 395.8	+ 9.4
7	42.23	+ 157.4	19.95	+ 85.0	+ 8.2	+ 149 2	+ 3 5
8	42.26	+ 7.0	15.74	+ 65.7	+ 6.3	+ 0.7	0.0
9	42.23	÷ 140.6	12.14	+ 48.7	+ 4.7	÷ 145.3	÷ 3.4
10	42.22	÷ 328.8	8.02	+ 28.1	+ 2.7	÷ 331.5	÷ 7.9

The concentration, c, of ammonium sulphate in all the experimental mixtures was about the same, and it was easily calculated — if we suppose the ammonium sulphate to be distributed proportionally on the whole volume of the experimental liquid — from the quantities weighed off to form the mixtures; c was in this way found to be about $0.358 \cdot n$. In our view, however, the ammonium sulphate will not be distributed in this way; the disperse phase, the egg-hydrate, is combined with a little ammonium sulphate, while by far the largest part is distributed proportionally in the dispersion medium which surrounds the egg-albumin. Now it is possible, by means of the investigations set forth in Paper V, to calculate that the volume of the disperse phase will come to 3.77 cc. per 100 cc. experimental liquid in the experiments put together in Table 22. The volume, V_a, of the dispersion medium will thus be 96.23 cc. per 100 cc. experimental liquid, and if we suppose the amount of ammonium sulphate to be equally distributed on this volume, the concentration, c, of the ammonium sulphate will — if we count with the slight amount of ammonium sulphate combined with the egg-albumin — be approximately $0.370 \cdot n$.

The details of this calculation are the following: In 100 cc. experimental liquid 42.24 milligram-equivalents protein nitrogen (Table 22, column 2) are present on an average, and the ammonium sulphate concentration is, as above stated, about $0.358 \cdot n$, which corresponds with $S = 2.4$ (S is the amount of ammonium sulphate per 100 g. water; see Table 32).

From the graphical sketches given in Paper V of the dependence between S on one hand and z or y, respectively, on the other, it results that z (the factor by which the weight of the protein nitrogen has to be multiplied to give the total weight of the disperse phase) and y (the factor by which the weight of the protein nitrogen has to be multiplied to give the weight of the ammonium sulphate combined with the disperse phase), if $S = 2.4$, have the values $z = 8.08$ and $y = 0.0257$.

Hence, the weight of the disperse phase in 100 cc. experimental liquid will be (expressed in g.):

$$\frac{42.24 \cdot 14.01 \cdot 8.08}{1000};$$

and if the specific gravity of the disperse phase is equal to 1.27 (the determination of this specific gravity will be described in a subsequent paper), the volume of the disperse phase will be (expressed in cc.):

$$\frac{42.24 \cdot 14.01 \cdot 8.08}{1000 \cdot 1.27} = 3.77.$$

The weight of the amount of ammonium sulphate combined with the disperse phase is (expressed in g.):

$$\frac{42.24 \cdot 14.01 \cdot 0.0257}{1000};$$

if we distribute this weight of ammonium sulphate on 100 cc., it will correspond to the concentration

$$\frac{42.24 \cdot 14.01 \cdot 0.0257}{1000 \cdot 66.08} \cdot \frac{1000}{100} = 0.002 \cdot n.$$

Hence, the volume, V_a, of the dispersion medium corresponding with 100 cc. experimental liquid will be:

$$V_a = 100 \div 3.77 = 96.23 \text{ cc.},$$

and the real concentration, c, of the ammonium sulphate in the dispersion medium surrounding the disperse phase will be

$$c = \frac{(0.358 \div 0.002) \cdot 100}{96.23} = 0.370 \cdot n.$$

As the real ammonium sulphate concentration, $0.370 \cdot n$, is thus a little higher than $0.358 \cdot n$, the figure from which we started in our calculations, we ought to recalculate with the real concentration as starting point. Now the concentration $0.370 \cdot n$ will correspond with $S = 2.47$, which, in turn, will answer to $z = 8.10$ and $y = 0.0262$. If we recalculate with these values, V_a and c will be found to attain practically the same values as above; therefore these values are used in the calculation of Table 22.

The quantities stated in Table 22, second and third columns, can be easily calculated from the quantities weighed off for the experimental mixtures. The fourth column contains the averages of the values of h found by the measurements of the hydrogen-ion concentration, and the fifth column contains the concentration of surplus acid of the dispersion medium, calculated from the values of h by means of the formula:

$$s = h\ f_s \div \frac{(x_{max})^2}{h \cdot f_s} \text{ (see section A, p. 76).}$$

It is easy from the concentration of surplus acid in the dispersion medium to calculate the whole amount of surplus acid, t', (expressed in cc. $^n/_{1000}$ acid) present in the volume, V_a, of the dispersion medium; this quantity is stated in the sixth column. The difference between the total amount of surplus acid or ammonia, t, in 100 cc. experimental liquid and t' is equal to the amount of surplus acid or base combined with the disperse phase. $t \div t'$, expressed in cc. $^n/_{1000}$, is given in the last but

one column, while the last column contains the amount of surplus acid or ammonia (in cc. $^{n}/_{1000}$) combined with one milligram-equivalent protein nitrogen and calculated from the values of $t \div t'$.

The series of experiments marked *E* stated in Table 22 forms a part of the main experiment, which consists of nine such series of experiments; the measurements of hydrogen-ion concentrations were in all the series executed by Miss Jenny Hempel For these experiments we used the same stock-solution of egg-albumin, marked D. Æ. 6. (prepared in 1915)[1], of which weighed quantities were mixed in proper amounts with weighed quantities of ammonium sulphate solution, titrated diluted sulphuric acid or ammonia and water, in such a way that the concentration of egg-albumin and ammonium sulphate was kept constant within each single series, but varied from one series to another. The calculation of the capacity to combine with acids was made in quite the same manner in the case of the other series as mentioned above for series *E*. The results of these calculations are set forth in Table 23 and graphically represented on the *main curve table*, which is reproduced on Fig. 10 on a much reduced scale. The hydrogen-ion concentration *h* is used as abscissa, while the amount, *b* (expressed in cc. $^{n}/_{1000}$), of sulphuric acid (+) or ammonia (÷) combined with one milligram-equivalent protein nitrogen is adopted as ordinate. Experiments belonging to the same series are marked on the curve table in the same way, and the mode of designation used in each series is stated at the top of Table 23.

From Table 23, and especially clearly from Fig. 10, it appears that it is possible to represent the Series *I* and *H*, which have about the same ammonium sulphate concentration (c = about $0.062 \cdot n$), by one and the same curve (I); the same applies to the Series *G*, *F* and *E* (c = about $0.363 \cdot n$, Curve II) and the Series *D*, *C* and *B* (c = about $1.417 \cdot n$, Curve III), while Series *A* ($c = 2.817 \cdot n$) demands its own curve, IV. This means — expressed in other terms — that, *if the ammonium sulphate con-*

[1]) The stock solution mixed with toluol was kept in ice in an ice-safe; for each series of experiments a proper quantity was filtered from the excess of toluol, after which the last remainder of toluol was removed by a current of hydrogen. After being filtered again and analysed, the solution was used for the preparation of the experimental mixtures.

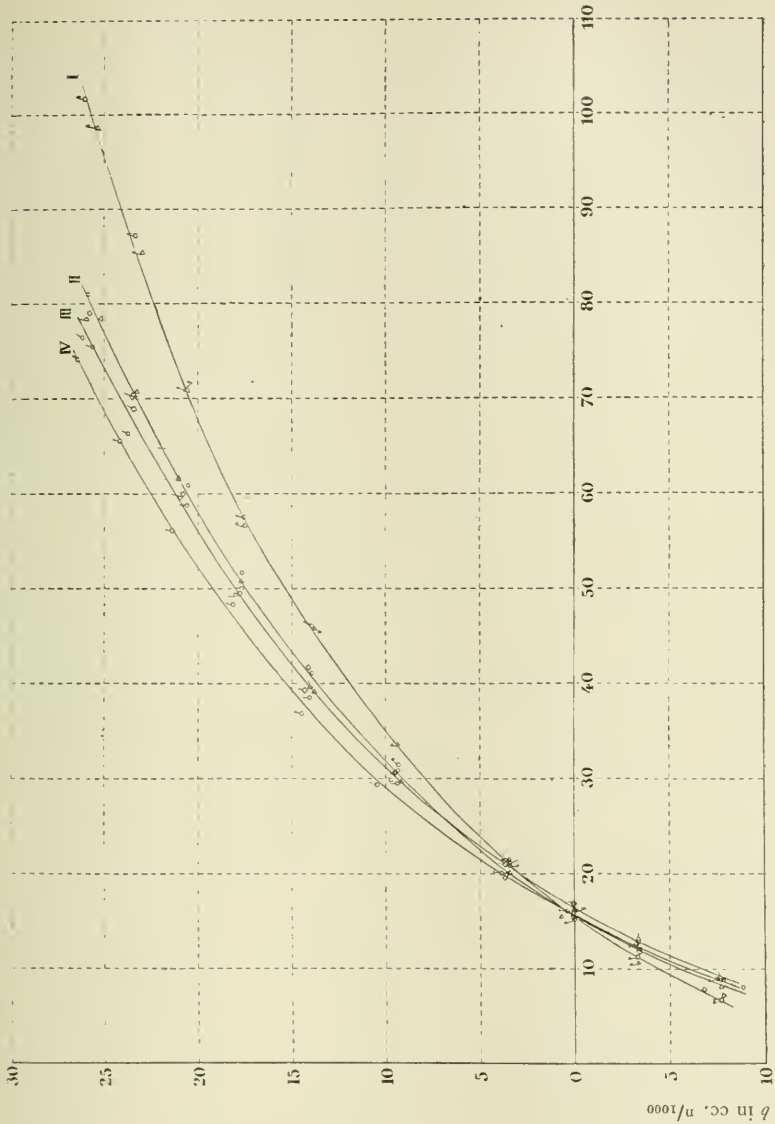

Fig. 10.

centration is constant, the capacity, b, to combine with acids is independent of the egg-albumin concentration, which — as shown by Table 23 — varies considerably within the series represented by the same curve.

From Fig. 10 is further seen that, at hydrogen-ion concentrations greater than about $15—16 \cdot 10^{-6}$, b is positive, that is, the egg-albumin is combined with surplus sulphuric acid; at

TABLE 23. **Capacity of Egg-albur**

Series of experiments:				
A	*B*	*C*	*D*	*E*
Mark:				
♂	♂	♂	♂	○
Ammonium sulphate concentration, :				
2.817 n	1.443 n	1.409 n	1.399 n	0.37
Number, e, of milligram-equivalents protein nitrogen in 100 cc. experimental liquids:				
about 10.5	about 31.7	about 10.5	about 4.2	about 4

Hydrogen-ion concentration, h, measured	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000	Hydrogen-ion concentration, h, measured	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000	Hydrogen-ion concentration, h, measured	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000	Hydrogen-ion concentration, h, measured	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000	Hydrogen-ion concentration, h, measured
$h \cdot 10^6$	$b = \frac{t \div t'}{e}$	$h \cdot 10^6$	$b = \frac{t \div t'}{e}$	$h \cdot 10^6$	$b = \frac{t \div t'}{e}$	$h \cdot 10^6$	$b = \frac{t \div t'}{e}$	$h \cdot 10^6$
74.19	+ 26.4	75.47	+ 25.7	78.40	+ 25.9	76.39	+ 26.1	80.91
65.67	24.1	66.27	23.7	70.15	23.5	68.86	23.4	70.17
56.10	21.4	58.80	20.6	59.62	21.0	59.66	20.9	60.91
48 31	18.1	49.97	17.7	49.44	17.9	—	—	51.76
36.82	14.5	38.64	14.1	39.63	14.1	39.33	14.3	41.09
29.32	10.5	29.39	9.4	30.60	9.5	29.91	9.7	30.74
20.14	3.8	21.50	3.5	21.18	3.5	20.98	3.6	19.95
16.06	0.4	16.85	0.0	16.76	0.1	17.13	0.0	15.74
12.37	÷ 3.2	12.75	÷ 3.4	13.08	÷ 3.4	12.59	÷ 3.3	12.14
8.91	÷ 7.6	8.81	÷ 7.8	8.97	÷ 7.7	9.01	÷ 7.5	8.02

combine with Acids.

	F	G	H	I
	△	□	⌀	◺
	0.365 n	0.354 n	0.065 n	0.059 n
	about 32.0	about 10.5	about 31.7	about 10.5

Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000 $b = \frac{t \div t'}{e}$	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000 $b = \frac{t \div t'}{e}$	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000 $b = \frac{t \div t'}{e}$	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000 $b = \frac{t \div t'}{e}$	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. n/1000 $b = \frac{t \div t'}{e}$
+ 25.8	78.46	+ 25.2	79.13	+ 25.8	101.7	+ 26.0	98.70	+ 25.3
23.2	70.69	23.3	70.48	23.4	87.24	23.4	85.44	22.9
20.5	61.58	21.1	59.99	20.8	70.86	20.6	70.85	20.5
17.7	50.83	17.7	49.51	17.8	57.50	17.5	56.71	17.5
13.9	39.22	13.8	41.74	14.1	45.08	13.9	45.93	13.8
9.4	29.69	9.2	30.68	9.5	31.49	9.3	33.57	9.4
3.5	20.06	3.5	19.70	3.6	20.83	3.5	21.43	3.5
0.0	15.51	0.0	15.48	0.1	15.23	0.0	16.06	0.0
÷ 3.4	12.09	÷ 3.3	11.85	÷ 3.4	11.19	÷ 3.2	10.55	÷ 3.4
÷ 7.9	8.10	÷ 7.7	7.92	÷ 7.7	6.82	÷ 7.7	7.15	÷ 7.8

lower hydrogen-ion concentrations, on the contrary, b is negative, that is, the egg-albumin is combined with surplus ammonia. The three curves intersect at the hydrogen-ion concentration $16 \cdot 10^{\div 6}$; the fourth curve, corresponding with $c = 1.417 \cdot n$, differs at hydrogen-ion concentrations lower than $25 \cdot 10^{\div 6}$ a little from the others; but the difference is not greater than may be easily accounted for by experimental errors. If we do not take this difference into consideration, it is evident from the curve table that *the amount of fixed surplus sulphuric acid (b positive) at a certain hydrogen-ion concentration is the greater as the ammonium sulphate concentration increases, and that the amount of fixed surplus ammonia (b negative) at a certain hydrogen-ion concentration likewise increases with the ammonium sulphate concentration.*

The results arrived at in the case of egg-albumin are thus analogous with those obtained for the simple ampholytes (see p. 112), and correspond exactly with the above theory of the capacity of ampholytes to combine with acids (see p. 106).

We must draw attention to yet another point. In the above calculations we have left out of consideration the dissociation of the egg-albumin into hydrogen- and anions and hydroxyl- and kations respectively. Hence, the curves represented in Fig. 10 are subject to a systematic error, which has the greater significance the nearer the hydrogen-ion concentration of the solution is to the isoelectric point of egg-albumin. Further, as proved in the theoretical section (see p. 106), Fig. 10 goes to show that the isoelectric reaction of egg-albumin, as defined above (see p. 106), must be $15-16 \cdot 10^{\div 6}$, because egg-albumin — under the incorrect assumptions mentioned — does not at this hydrogen-ion concentration combine either with surplus sulphuric acid or ammonia. That the real isoelectric point of egg-albumin is situated precisely at this hydrogen-ion concentration, we shall prove in another way in the concluding part of the present paper (Section D.).

b. Is the Capacity to combine with Acids under otherwise equal circumstances the same for all Egg-albumin solutions?

The next question which we had to answer was the following: Do the results obtained by the egg-albumin solution used for our main experiment apply to other egg-albumin solutions as well? In other words, do the found numerical expression of the

capacity to combine with acids and the graphical representation of it apply to egg-albumin solutions in general, so that it will be possible to use the capacity to combine with acids for the characterisation of this protein?

In order to answer this question, we have in the Tables 24, 25 and 26 placed together some series of experiments in which we used egg-albumin solutions prepared at different periods. The experiments[1] were made by exactly the same method as described under the main experiment, and the calculation (by means of the measured hydrogen-ion concentration, h) of the capacity, b, to combine with acids was made as stated above. In the Tables 24, 25 and 26 we have set forth, besides the value of b thus calculated, the corresponding value of b as found by means of the curve table If the ammonium sulphate concentration did not correspond with any of the curves plotted on Fig. 10, it was possible to find the value of b by interpolation between two curves or, if necessary, by a less exact extrapolation

For all the experiments stated in Table 24 we used an egg-albumin solution (marked P_{IV}) which had been prepared in the year 1913 and dialysed by the usual method. The ammonium sulphate concentration was in all the experiments the same, namely $0.35 \cdot n$, corresponding with one of the curves in Fig. 10; the egg-albumin concentration, on the contrary, was different, and the experiments may be arranged in three series with concentrations of protein in the ratio 1 : 2 : 3. The table shows that the accordance between the value of b as calculated from the measurement and that found by means of the main curve table (Fig. 10) is excellent, since the difference never reaches 1 cc. $^n/_{1000}$.

All the experiments stated in Table 25 were made with an egg-albumin solution (marked P_{III}) which had likewise been prepared in 1913. In these experiments we varied partly the concentration of protein (within rather narrow limits), partly the concentration of ammonium sulphate. Also in this case the accordance between the value of b calculated from the measurement and that found by means of the curve table (Fig. 10) is unexceptionable.

The experiments in Table 26 were made with an egg-albumin solution (marked P_V) prepared in 1914. In these experiments

[1] The measurings of the hydrogen-ion concentration were made by Mr. S. Palitzsch.

TABLE 24.

Capacity of Egg-albumin to combine with Acids.

For all the experiments an egg-albumin solution was used which was prepared in 1913, dialysed and marked PIV. The number, e, of milligram-equivalents protein nitrogen in 100 cc. experimental liquid was in experiment Nos. 1 to 6: about 8.2; in Nos. 7 to 22: abt. 16.3; in Nos. 23 to 33: abt. 24.5.

The ammonium sulphate concentration, c, was in all experiments abt. $0{,}35 \cdot n$.

No. of exp.	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. $n/1000$ $b = \frac{t \div t'}{e}$ calculated from measurement	derived from curve (Fig. 10)	No. of exp.	Hydrogen-ion concentration, h, measured $h \cdot 10^6$	Amount of sulphuric acid (+) or ammonia (÷) combined with one milligr.-equivalent protein nitrogen in cc. $n/1000$ $b = \frac{t \div t'}{e}$ calculated from measurement	derived from curve (Fig. 10)
1	77.93	+ 25.2	+ 25.2	18	12.81	÷ 2.9	÷ 2.6
2	46.82	+ 17.1	+ 16.3	19	12.32	÷ 2.9	÷ 3.1
3	27.73	+ 8.3	+ 7.9	20	10.07	÷ 5.3	÷ 5.4
4	20.38	+ 3.8	+ 3.7	21	9.89	÷ 5.3	÷ 5.7
5	14.84	÷ 0.8	÷ 0.6	22	8.08	÷ 7.7	÷ 8.0
6	9.64	÷ 5.4	÷ 6.0	23	68.79	+ 23.1	+ 23.0
7	68.24	+ 22.6	+ 22.8	24	54.79	+ 18.5	+ 19.0
8	59.05	+ 20.4	+ 20.3	25	39.85	+ 13.9	+ 13.6
9	44.71	+ 15.9	+ 15.5	26	30.63	+ 9.1	+ 9.4
10	33.55	+ 11.2	+ 10.6	27	25.92	+ 6.8	+ 7.0
11	29.02	+ 8.9	+ 8.6	28	22.61	+ 4.4	+ 5.1
12	24.76	+ 6.6	+ 6.4	29	18.09	+ 2.0	+ 1.9
13	20.96	+ 4.2	+ 4.1	30	15.04	÷ 0.4	÷ 0.5
14	17.84	+ 1.9	+ 1.8	31	12.51	÷ 2.8	÷ 2.9
15	15.55	÷ 0.5	÷ 0.1	32	10.06	÷ 5.2	÷ 5.4
16	15.05	÷ 0.5	÷ 0.5	33	8.20	÷ 7.6	÷ 7.8
17	14.78	÷ 0.5	÷ 0.7				

TABLE 25.

Capacity of Egg-albumin to combine with Acids.

In all the experiments we used a dialysed solution of egg-albumin prepared in 1913 and marked P III.

No. of exp.	Concentration of ammonium sulphate $c \cdot {}^{n}/_{1}$	Number, e, of milligram-equivalents protein-nitrogen in 100 cc. experimental liquid	Hydrogen-ion concentration, h, measured	Amount of sulphuric acid (+) or ammonia (÷) in cc. $^{n}/_{1000}$ combined with one milligram-equivalent protein nitrogen $b = \frac{t \div t'}{e}$	
				calculated from the measurement	deduced from the curve table (Fig. 10)
	c		$h \cdot 10^{6}$		
1	2.004	9.98	18.66	+ 1.9	+ 2.3
2	,,	,,	15.80	+ 0.1	÷ 0.2
3	,,	,,	13.89	÷ 1.7	÷ 1.7
4	,,	,,	10.45	÷ 5.3	÷ 5.1
5	1.003	,,	18.63	+ 2.5	+ 2.1
6	,,	,,	16.94	+ 0.6	+ 0.8
7	,,	,,	14.61	÷ 1.3	÷ 1.4
8	0.501	,,	19.04	+ 2.8	+ 2.6
9	,,	,,	17.15	+ 0.9	+ 1.3
10	,,	,,	14.43	÷ 1.0	÷ 1.2
11	0.351	15.57	60.90	+ 20.5	+ 20.8
12	0.376	9.98	19.16	+ 2.9	+ 2.9
13	0.379	14.97	17.88	+ 1.9	+ 1.8
14	0.376	9.98	17.23	+ 1.0	+ 1.4
15	0.379	14.97	16.78	+ 0.6	+ 1.1
16	0.382	19.96	15.10	÷ 0.6	÷ 0.5
17	0.376	9.98	14.98	÷ 0.9	÷ 0.6
18	0.379	14.97	14.76	÷ 0.7	÷ 0.8
19	0.376	9.98	14.69	÷ 0.9	÷ 0.8
20	0.379	14.97	13.76	÷ 2.0	÷ 1.8
21	,,	,,	11.76	÷ 3.3	÷ 2.9
22	0.252	9.98	19.94	+ 3.0	+ 3.3
23	,,	,,	17.04	+ 1.1	+ 1.1
24	,,	,,	14.35	÷ 0.8	÷ 1.0
25	,,	,,	10.38	÷ 4.6	÷ 4.9
26	0.016	,,	22.56	+ 3.4	(< + 4.5) [1]
27	,,	,,	9.62	÷ 4.4	> ÷ 4.7) [1]

[1] This figure corresponds with the curve of the lowest ammonium sulphate concentration, $c = 0.062$ (Fig. 10).

TABLE 26. **Capacity of Egg-albumin to combine with Acids.**

In all the experiments we used a dialysed solution of egg-albumin prepared in 1914 and marked Pv. The number, c, of milligram-equivalents protein nitrogen in 100 cc. experimental liquid was in experiments Nos. 1—13: 20.460; Nos. 14—24: 2.046; Nos. 25—26: 1.023.

No. of exp.	Concentration of ammonium sulphate $c \cdot n/_1$	Hydrogen-ion concentration, h, measured	Amount of sulphuric acid (+) or ammonia (÷) in cc. $n/_{1000}$ combined with one milligram-equivalent protein nitrogen $b = \frac{t \div t'}{e}$		No. of exp.	Concentration of ammonium sulphate $c \cdot n/_1$	Hydrogen-ion concentration, h, measured	Amount of sulphuric acid (+) or ammonia (÷) in cc. $n/_{1000}$ combined with one milligram-equivalent protein nitrogen $b = \frac{t \div t'}{e}$	
			calculated from the measurement	deduced from the curve table (Fig. 10)				calculated from the measurement	deduced from the curve table (Fig. 10)
	c	$h \cdot 10^6$				c	$h \cdot 10^6$		
1	2.803	25.98	+ 8.8	+ 8.1	14	2.750	17.61 [1]	+ 4.4	+ 1.8
2	,,	14.76	÷ 0.4	÷ 0.8	15	,,	12.44	÷ 2.2	÷ 3.4
3	,,	8.35	÷ 7.8	÷ 8.2	16	1.375	19.35	+ 4.6	+ 2.3
4	1.053	14.96	÷ 0.3	÷ 1.0	17	,,	12.52	÷ 2.3	÷ 3.8
5	0.702	28.75	+ 9.1	+ 8.6	18	0.344	22.21 [1]	+ 5.7	+ 4.9
6	,,	15.10	÷ 0.3	÷ 0.7	19	,,	19.66	+ 6.2	+ 3.2
7	0.353	29.31	+ 9.2	+ 8.7	20	,,	12.09	÷ 1.9	÷ 3.3
8	,,	28.42	+ 9.2	+ 8.3	21	,,	11.98	÷ 1.9	÷ 3.4
9	,,	15.01	÷ 0.3	÷ 0.6	22	,,	11.65	÷ 1.8	÷ 3.7
10	,,	14.85	÷ 0.3	÷ 0.6	23	0.069	22.23	+ 7.3	+ 4.1
11	,,	14.81	÷ 0.3	÷ 0.7	24	,,	11.05	÷ 1.2	÷ 3.5
12	0.143	32.70	+ 9.3	+ 9.4	25	0.343	16.77	+ 3.9	+ 0.9
13	,,	14.14	÷ 0.2	÷ 1.0	26	,,	10.42	÷ 3.0	÷ 5.1

[1]) The measurement is marked in the experimental journal as being not quite certain.

we varied extensively both the concentration of ammonium sulphate and that of protein. The table shows that, whilst the accordance between the calculated and found values of b is satisfactory in the experiments where the protein concentration is high ($e = 20.460$), it is not so in the experiments where the protein concentration is very low ($e = 2.046$ or $e = 1.023$). In these latter groups of experiments the value of b calculated from the measurements is markedly greater (the numerical value consequently smaller, if the sign is negative) than the value found from the curve table. As the hydrogen-ion concentrations in these experiments correspond more or less with the isoelectric point of the egg-albumin, the abnormal result just referred to is doubtless attributable to the fact that the curve table (Fig. 10) is based on experiments made with greater concentrations of protein and irrespective of the dissociation of the egg-albumin into hydrogen- and anions and into hydroxyl- and kations, respectively. We have already in a previous section (see p. 106) discussed this question in detail, and have shown that, at the isoelectric point and in the neighbourhood of the same, it is not permissible to leave out of consideration the dissociation of the ampholyte with formation of hydrogen- or hydroxyl-ions, respectively; and we have further shown that the magnitude of the term of correction relating to this dissociation depends on the ampholyte concentration in such a way that b (reckoned with sign) must attain a value higher in proportion as the ampholyte concentration is lower. *The difference found may thus be accounted for in full accordance with our theoretical assumptions.*

A closer examination of Table 26 will now show that, also in the case of those experiments where the concentration of protein is high, the values of b calculated from the measurements are generally higher than those deduced from the curve table, although the differences never come to 1 cc. $^{n}/_{1000}$. Thus, this sample of egg-albumin, as compared with the two preceding samples, does not show quite the same capacity to combine with acids as that on which the main experiment was made; yet the difference is so small that it may very well be due to experimental errors, of which we may here especially draw attention to that arising from a not quite exact determination of the amount of ammonia in the dialysed egg-albumin solution. If we put the limit of the experimental errors equal to 1 cc. $^{n}/_{1000}$ in

the value of b, *the normal egg-albumin solutions examined by us have shown the same capacity to combine with acids, and we consider this fact as a proof of the theory that egg-albumin solutions prepared in the manner described in the preceding paper always contain one and the same substance with the properties characteristic of it.*

The case must of course be different if the egg-albumin solution has begun to putrefy, or if a decomposition has taken place in another way, for instance by keeping too long in the presence of an excess of strong acids or bases. We must in this case expect an increased capacity to combine with acids or bases, because acid- as well as base-binding groups may be set free by the splitting up. This fact has been experimentally demonstrated only in the case of a single sample of egg-albumin: — we have made some measurements of hydrogen-ions and have from these calculated b in some mixtures prepared with the dialysed egg-albumin solution mentioned in Paper I (see p. 38). This egg-albumin solution, after removal of the toluol, had a distinct but very inconsiderable putrid smell. In all the experiments the ammonium sulphate concentration, c, was equal to $0.354 \cdot n$, and the number, e, of milligram-equivalents protein nitrogen in 100 cc. experimental liquid was equal to 14.2. The results of the measurings, which are stated below, show that the capacity to combine with acids, as might have been expected, was greater than it is normally.

$h \cdot 10^6$	b (calculated from the measurement)	b (found by means of the curve table)
61.95	+ 26.23	+ 21.70
54.77	23.64	19.00
47.76	21.00	16.65

c. Determination, by means of the Main Curve Table, of the Amount and the Distribution of Surplus Sulphuric Acid in Egg-albumin solutions containing Ammonium Sulphate.

We shall now revert to the question made in the introduction to this paper as to the possibility of determining, by means of hydrogen-ion measurement, both *the whole present amount of "surplus" sulphuric acid or ammonia and its distribution between the egg-albumin and the dispersion medium.* We have in the preceding section seen that this question must be answered in the affirmative, inasmuch as the main curve table (Fig. 10) permits us to read b if h has been measured, — provided of course that the composition of the solution is known. Since further — as mentioned above —, if h is measured, it is possible to calculate the amount of surplus sulphuric acid or ammonia contained in

the dispersion medium, we have all the data necessary for answering the question under discussion. The foundation on which the main curve table (Fig. 10) is based is, however, as repeatedly mentioned, not quite correct, since in the calculation we have not taken into consideration the dissociation of the egg-albumin into hydrogen- and anions and into hydroxyl- and kations, respectively, and it will now be of some interest to examine what influence this deficiency in the elaboration of the curve table exerts on the exactness of the results obtained by means of the table. We shall in this section discuss the matter in some detail.

It is hardly possible to accomplish a quantitative treatment of the question; but it is easy to judge of the qualitative significance of the source of error referred to, if we consider that the egg-albumin is an ampholyte of markedly acid reaction, so that, practically, we need not take into consideration the dissociation into hydroxyl- and kations. The error then arises from the circumstance that in calculating the concentration, s, of surplus acid in the dispersion medium from the equation

$$s = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s}$$

by means of the measured value of h, we have not taken into consideration the fact that some of the hydrogen ions which give the hydrogen-ion concentration h originate in the dissociation of the egg-albumin into hydrogen- and anions and therefore ought not to be included in the calculation of s. Thus the error manifests itself in the circumstance that the values of s (reckoned with sign) and of t' (calculated by means of s), which represents the amount of surplus acid present in the dispersion medium of 100 cc. experimental liquid, are calculated too high. Consequently, the amount of acid combined with the egg-albumin is calculated too low, and it is evident that in the value of b calculated by means of the equation

$$b = \frac{t \div t'}{e},$$

where t designates the total amount of surplus acid present in 100 cc. experimental liquid and e the number of milligram-equivalents of protein nitrogen in the same volume of experimental liquid, an error in t' will be, comparatively, of the greater importance the more $t \div t'$ approaches to zero and the smaller e is.

The result is then, that *the values of b (the capacity to combine with acids, reckoned with sign) deduced from the curve table (Fig. 10) are always too low, and the error is comparatively greater the more b approaches zero and the lower the protein concentration of the solution is. On the other hand, the above mode of calculating t' (the content of surplus acid of the dispersion medium) will give too high a value, so that the total content of surplus acid in the solution will be correct, since it is equal to the sum of the surplus acid combined with the egg-albumin and that existing in the dispersion medium. This conclusion will, however, only be correct in the case of solutions with protein concentrations corresponding fairly with those upon which the curve table (Fig. 10) is based. As above explained, at very low concentrations of protein we are not justified in considering b as independent of the concentration of protein, which we did when constructing the curve table. The latter gives in such cases rather low values of b, and hence the total amount of surplus acid in the solution will also be found too low.*

d. Determination, without using the main curve table, of the whole contents of Surplus Sulphuric Acid in Egg-albumin solutions containing Ammonium Sulphate.

It is possible to determine the total amount of surplus acid by a more direct proceeding than that described in *Section c*. This second method always gives a correct result; but it affords no information concerning the distribution of the surplus acid between the two phases of the solution, and is on the whole more circumstantial than the method mentioned above.

In order to elucidate this state of things more thoroughly, we have in Table 27 set forth all the experiments which we have carried out with an ammonium sulphate concentration of about $0.36 \cdot n$, and most of which have been used for the calculations made in the preceding sections. In the table experiments are set forth from 11 series of experiments with different samples of egg-albumin. As appears from the uppermost part of the table, the concentration of the protein, which is a function of c, varies widely in the different series; but the ammonium sulphate concentration is nearly the same in all the series. In the table we have stated the hydrogen-ion concentration, h, found for each

experimental mixture, the amount t, expressed in cc. $^{n}/_{1000}$, of surplus acid (+) or ammonia (÷) present in 100 cc. of this mixture, and the quantity q calculated therefrom; this quantity represents the amount, in cc. $^{n}/_{1000}$, of surplus sulphuric acid (+) or ammonia (÷) per milligram-equivalent of protein nitrogen (see Table 27 and Fig. 11).

If h is plotted as abscissa against q as ordinate on a graphical sketch of the experimental results set forth in Table 27, we shall obtain eleven curves corresponding with the eleven experimental series; five of these curves are reproduced in Fig. 11, and represent the following values of e: 1.023; 2.046; 10.52; 16.33; 42.24[1]). It is now possible from each curve graphically to deduce the hydrogen-ion concentration, h, of the corresponding egg-albumin solution, if the amount of surplus sulphuric acid q per milligram-equivalent of protein nitrogen has a given value. In Table 28 we have set forth the values of h deduced graphically in this way for the different values of q (from + 10 to ÷ 6) in the eleven series of experiments, with different concentrations of protein (different values of e), here in question. Besides we have — as the second horizontal column — stated the hydrogen-ion concentration of a pure aqueous solution of ammonium sulphate of the concentration 0.36 · n. The hydrogen-ion concentration of this solution, which with the value $e = 0$ forms the natural starting point of the other solutions, is calculated from formula (7), given in Section A (p. 76), which, if $s = 0$, assumes the form

$$h \cdot f_s = x_{max}$$

or

$$h = \sqrt{c \cdot \frac{k_w}{k_m} \cdot \frac{\beta}{f_s}}$$

It appears from Table 28 (p. 141) that the hydrogen-ion concentration at a constant ammonium sulphate concentration will rise as the protein concentration increases. This will be the case both with pure egg-albumin ($q = 0$) and egg-albumin containing surplus sulphuric acid (q positive) or surplus ammonia (q negative); but it is evident from the table that the increase of the hydrogen-ion concentration — as was to be expected — is the stronger the greater q is. If q is *negative* — that is, if the egg-albumin

[1] We have in Fig. 11 only given the 5 curves above mentioned, as the curves of the relatively strong egg-albumin solutions are situated so very near each other.

TABLE 27. **Dependence of the Hydrogen-ion concentration on**

The ammonium sulphate concentration, c,

Experiments Nos. 1 to 10: Egg-albumin solution: Main experiment, E; $c = 0.$

,, ,, 11 ,, 20: ,, ,, ,, ,, F; $c = 0.$

,, ,, 21 ,, 30: ,, ,, ,, ,, G; $c = 0.$

,, ,, 31 ,, 35: ,, ,, PIII ; $c = 0.$

,, ,, 36 ,, 38: ,, ,, PIII ; $c = 0.$

,, ,, 39 ,, 50: ,, ,, PIV ; $c = 0.$

,, ,, 51 ,, 63: ,, ,, PIV ; $c = 0.$

,, ,, 64 ,, 70: ,, ,, PIV ; $c = 0.$

,, ,, 71 ,, 73: ,, ,, PV ; $c = 0.$

,, ,, 74 ,, 76: ,, ,, PV ; $c = 0.$

,, ,, 77 ,, 79: ,, ,, PV ; $c = 0.$

Number of experiment	Hydrogen-ion concentration, h, measured	Total amount, t, of surplus sulphuric acid (+) or ammonia (÷) in cc. $^n/_{1000}$ present in 100 cc. experimental liquid	Amount, q, of surplus sulphuric acid (+) or ammonia, (÷) in cc. $^n/_{1000}$ present per milligram-equivalent protein nitrogen	Number of experiment	Hydrogen-ion concentration, h, measured	Total amount, t, of surplus sulphuric acid (+) or ammonia (÷) in cc. $^n/_{1000}$ present in 100 cc. experimental liquid	Amount, q, of surplus sulphuric acid (+) or ammonia (÷) in cc. $^n/_{1000}$ present per milligram-equivalent protein nitrogen
	$h \cdot 10^6$	t	$q = \frac{t}{c}$		$h \cdot 10^6$	t	$q = \frac{t}{c}$
1	80.91	+ 1125.8	+ 26.61	21	79.13	+ 305.5	+ 29.01
2	70.17	1009.9	23.93	22	70.48	276.3	26.24
3	60.91	893.4	21.14	23	59.99	244.6	23.23
4	51.76	768.2	18.19	24	49.51	208.2	19.77
5	41.09	606.3	14.35	25	41.74	166.2	15.78
6	30.74	408.7	9.68	26	30.68	113.1	10.76
7	19.95	157.4	3.73	27	19.70	46.3	4.41
8	15.74	7.0	0.17	28	15.48	7.06	0.67
9	12.14	÷ 140.6	÷ 3.33	29	11.85	÷ 31.1	÷ 2.95
10	8.02	÷ 328.8	÷ 7.79	30	7.92	÷ 78.0	÷ 7.42
11	78.46	+ 843.3	+ 26.26	31	17.88	+ 35.84	+ 2.39
12	70.69	766.4	24.28	32	16.78	15.84	1.06
13	61.58	679.0	21.52	33	14.76	÷ 4.16	÷ 0.28
14	50.83	580.6	18.39	34	13.76	÷ 24.16	÷ 1.61
15	39.22	461.5	14.35	35	11.76	÷ 44.16	÷ 2.95
16	29.69	308.8	9.60	36	19.16	+ 37.04	+ 3.71
17	20.06	121.0	3.76	37	17.23	17.04	1.71
18	15.51	7.0	0.22	38	14.83	÷ 2.96	÷ 0.30
19	12.09	÷ 102.2	÷ 3.18	39	68.79	+ 596.2	+ 24.33
20	8.10	÷ 242.3	÷ 7.55	40	54.79	476.19	19.44

tal amount of Sulphuric Acid in Egg-albumin solutions.

all the experimental mixtures about 0.36 · n.

mber of milligr.-equiv. protein nitrogen in 100 cc. experimental liquid, $e = 42.24$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 31.96$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 10.52$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 14.97$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 9.98$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 24.50$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 16.33$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 8.17$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 20.46$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 2.046$.
,, ,, ,, ,, ,, ,, ,, ,, ,, , $e = 1.023$.

Number of experiment	Hydrogen-ion concentration, h, measured	Total amount, t, of surplus sulphuric acid (+) or ammonia (÷) in cc. $^n/_{1000}$ present in 100 cc. experimental liquid	Amount, q, of surplus sulphuric acid (+) or ammonia (÷) in cc. $^n/_{1000}$ present per milligram-equivalent protein nitrogen	Number of experiment	Hydrogen-ion concentration, h, measured	Total amount, t, of surplus sulphuric acid (+) or ammonia (÷) in cc. $^n/_{1000}$ present in 100 cc. experimental liquid	Amount, q, of surplus sulphuric acid (+) or ammonia (÷) in cc. $^n/_{1000}$ present per milligram-equivalent protein nitrogen
	$h \cdot 10^6$	t	$q = \frac{t}{e}$		$h \cdot 10^6$	t	$q = \frac{t}{e}$
41	39.85	+ 356.19	+ 14.54	61	9.98	÷ 82.24	÷ 5.04
42	30.63	236.19	9.64	62	8.08	÷ 122.24	÷ 7.49
43	25.92	176.19	7.19	63	4.89	÷ 202.24	÷ 12.38
44	22.61	116.19	4.74	64	77.93	+ 239.33	+ 29.29
45	18.09	56.19	2.29	65	46.82	159.33	19.50
46	15.04	÷ 3.81	÷ 0.16	66	27.73	79.33	9.71
47	12.51	÷ 63.81	÷ 2.60	67	20.38	39.33	4.81
48	10.06	÷ 123.81	÷ 5.05	68	14.84	÷ 0.67	÷ 0.08
49	8.20	÷ 183.81	÷ 7.50	69	9.64	÷ 40.67	÷ 4.98
50	6.04	÷ 243.81	÷ 9.95	70	5.84	÷ 80.67	÷ 9.87
51	68.24	+ 397.76	+ 24 36	71	28.87	+ 200.78	+ 9.81
52	59.05	357.76	21.91	72	14.88	+ 0.78	0.04
53	44.71	277.76	17.01	73	7.33	÷ 159.22	÷ 7.78
54	33.55	197.76	12.11	74	20.93	+ 20.89	+ 10.21
55	29.02	157.76	9.66	75	11.87	0.89	0.43
56	24.76	117.76	7.21	76	6.55	÷ 15.11	÷ 7.39
57	20.96	77.76	4.76	77	16.77	+ 10.89	+ 10.65
58	17.84	37.76	2.31	78	10.42	0.89	0.87
59	15.13	÷ 2.24	÷ 0.14	79	6.16	÷ 7.11	÷ 6.95
60	12.57	÷ 42.24	÷ 2.59				

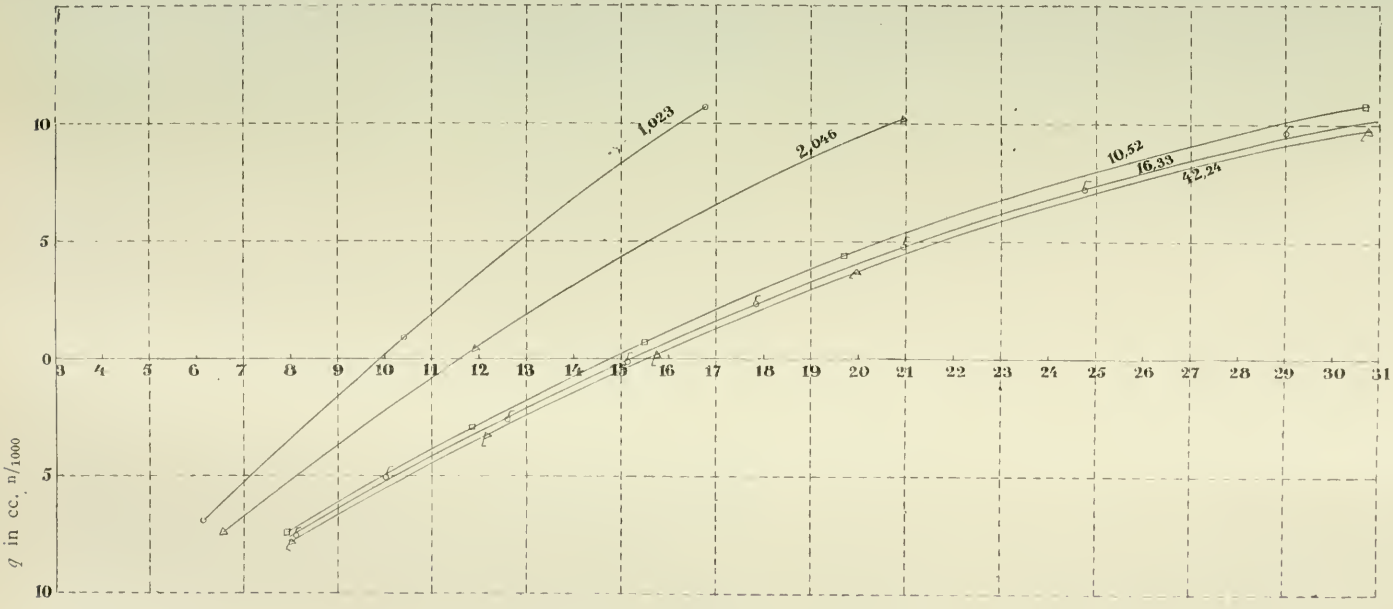
1,023
2,046
10,52
16,33
42,24
10
5
0
5
10
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
q in cc. n/1000
h · 10⁶

Fig. 11.

TABLE 28.

Hydrogen-ion concentration, h, of an ammonium sulphate containing egg-albumin solution (ammonium sulphate concentration, c = about $0.36 \cdot n$) which in 100 cc. experimental liquid contains e milligram-equivalents of protein nitrogen and an amount of surplus sulphuric acid (+) or ammonia (÷) of q cc. $^{n}/_{1000}$ per milligram-equivalent protein nitrogen. In the table the quantity $h \times 10^6$ is stated.

e	$q =$ + 10	+ 8	+ 6	+ 4	+ 2	0	÷ 2	÷ 4	÷ 6
0	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64	3.64
1.023	16.22	14.80	13.48	12.25	11.06	9.90	8.78	7.67	6.62
2.046	20.67	18.40	16.48	14.72	13.10	11.57	10.15	8.77	7.46
8.17	28.30	24.90	22.05	19.50	17.18	14.95	12.86	10.90	9.07
9.98	—	—	—	19.60	17.30	15.25	—	—	—
10.52	28.75	25.00	21.85	19.20	16.87	14.78	12.77	10.85	9.09
14.97	—	—	—	—	17.48	15.25	13.12	—	—
16.33	30.35	26.10	22.70	19.90	17.45	15.22	13.10	11.14	9.32
20.46	29.42	25.53	22.42	19.65	17.08	14.85	12.82	10.91	9.00
24.50	31.40	27.50	23.90	20.65	17.75	15.22	13 13	11 17	9.38
31.96	30.65	26.75	23.32	20.38	17.70	15.28	13.25	11.30	9.47
42.24	—	26.65	23.20	20.32	17.84	15.54	13.40	11.43	9.56

contains surplus ammonia —, the increase of the hydrogen-ion concentration will consequently be the smaller the greater the numerical value of q is, and at a proper negative value of q the hydrogen-ion concentration will be independent of the protein concentration and be equal to that of the pure ammonium sulphate solution. At still greater negative values of q the hydrogen-ion concentration will decrease with increasing protein concentration.

On examining the vertical columns of Table 28 we do not find, as above supposed, a continual increase in the values of h with rising e; the differences, however, are never greater than may easily be accounted for as experimental errors, each single value of h being found from the corresponding curve and independent of the other values. The differences within each single vertical column therefore give an idea of the comparative exactness with which these measurements were accomplished, though executed at different times with different egg-albumin solutions and by two different experimenters. We have, making a compensating of these differences, plotted the results of Table 28 on Fig. 12,

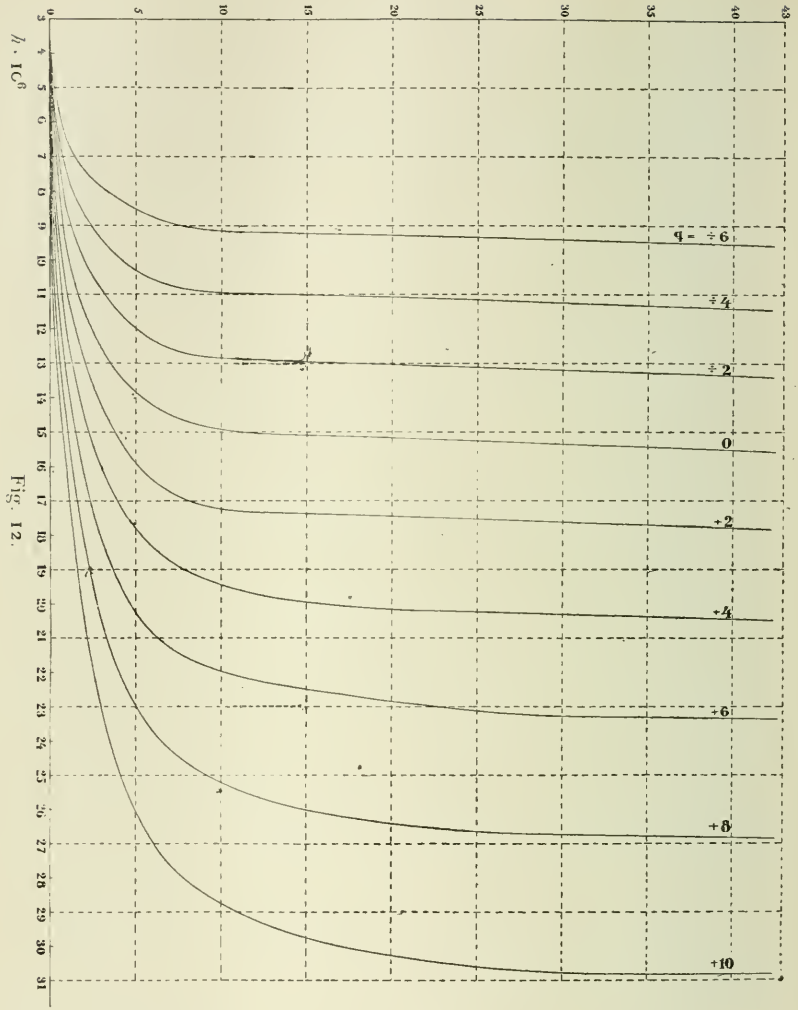

Fig. 12.

where the hydrogen-ion concentration h, is used as abscissa, and c — which is a measure of the egg-albumin concentration — is used as ordinate.

Each single curve in Fig. 12 corresponds with a certain value of q, and it will be easily seen that this curve table may be used for the determination of the whole content of surplus sulphuric acid or ammonia in a solution having an *ammonium sulphate concentration* $c = about\ 0{,}36 \cdot n$ and a known protein concentration, and the hydrogen-ion concentration of which has been measured. If thus we have a solution with an egg-albumin concentration corre-

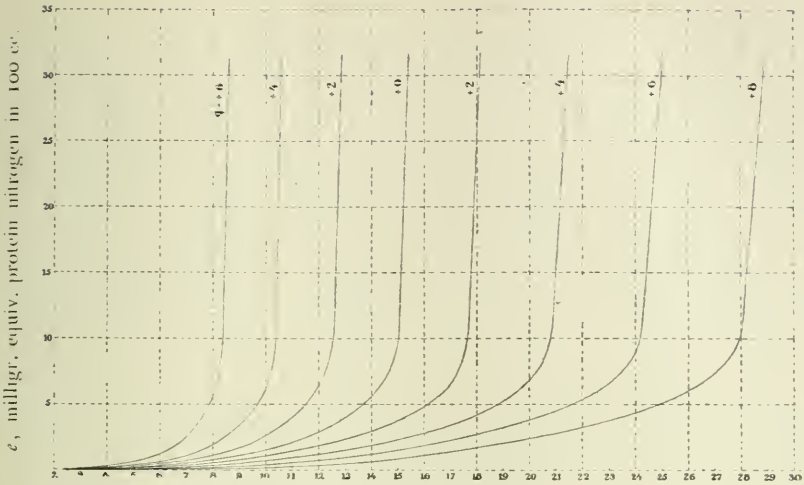

Fig. 13.

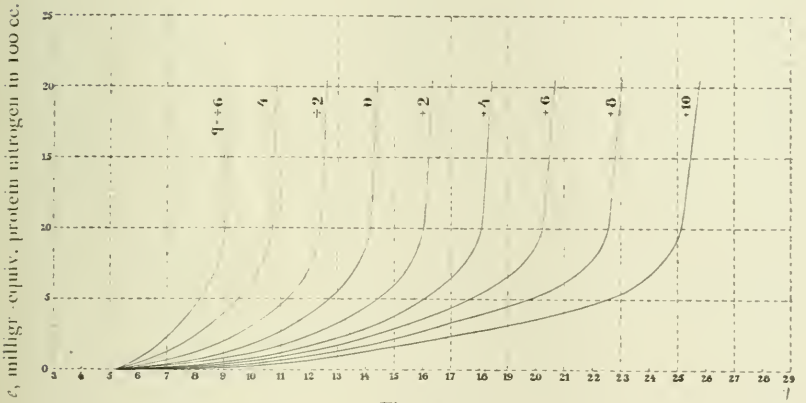

Fig. 14.

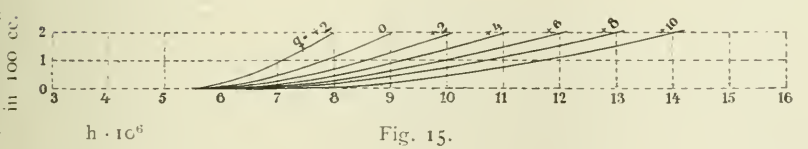

Fig. 15.

sponding to $e = 20$, and if we find the hydrogen-ion concentration equal to $19 \cdot 10^{\div 6}$, the curve table shows that q must be situated between $\div 2$ and $+ 4$. The curve corresponding with $q = 2$ gives (if $e = 20$) $h = 17.45 \cdot 10^{\div 6}$, while the curve $q = 4$ gives $h = 20.16 \cdot 10^{\div 6}$. By means of interpolation we shall then have (if $h = 19.00 \cdot 10^{\div 6}$) $q = + 3.14$, which means that an am-

monium-sulphate-containing egg-albumin solution, with an ammonium sulphate concentration of about $0.36 \cdot n$, with protein concentration corresponding to $c = 20$ and with hydrogen-ion concentration $19.00 \cdot 10^{\div 6}$, will contain an amount of surplus sulphuric acid equal to 3.14 cc. $^{n}/_{1000}$ per milligram-equivalent of protein nitrogen, consequently in 100 cc. solution altogether $20 \cdot 3.14 = 62.8$ cc. of $^{n}/_{1000}$ surplus sulphuric acid.

The bunch of curves represented in Fig. 12 applies only to the concentration of ammonium sulphate, $c =$ about $0.36 \cdot n$; the whole bunch of curves thus corresponds with the single curve which on the main curve table (Fig. 10) represents this concentration of ammonium sulphate. In order to be able to use the method above described also for the determination of the total amount of surplus sulphuric acid or ammonia in cases where the concentration of ammonium sulphate is very different from $0.36 \cdot n$, we must therefore have some similar bunches of curves to that given in Fig. 12, but corresponding with other concentrations of ammonium sulphate. Now the concentration of ammonium sulphate has proved to be of little consequence in the case of low values of q, and it is just in such cases that the use of the main curve table (Fig. 10) may give rise to the greatest errors. Therefore we have not considered it necessary to reproduce all the bunches of curves which we have, in quite a similar manner to the above, constructed for the different concentrations of ammonium sulphate, but confine ourselves to setting forth the bunches of curves corresponding with $c = 0.064 \cdot n$ (Fig. 13), $c = 2.79 \cdot n$ (Fig. 14) and $c = 4.12 \cdot n$ (Fig. 15). It will be seen that all four bunches of curves, of which the three last mentioned are constructed on the basis of considerably fewer experiments than the first (Fig. 12), have exactly the same character.

In order to elucidate the influence of the concentration of ammonium sulphate, we shall only mention that, in quite the same way as above, it appears from Fig. 13 and Fig. 14 that, if $c = 20$ and $h = 19.00 \cdot 10^{\div 6}$, q will be equal to $+ 2.19$ if $c = 0.064 \cdot n$, and q equal to $+ 4.50$ if $c = 2.79 \cdot n$.

A comparison of these values:

$c = 0.064 \cdot n$	$q = + 2.69$
$c = 0.36 \cdot n$	$q = + 3.14$
$c = 2.79 \cdot n$	$q = + 4.50$

will show that even a very considerable alteration in the value of c gives only a slight alteration in the size of q. In the cases where we have used this method for determining the total amount of surplus sulphuric acid or ammonia present, we have therefore considered these three bunches of curves as sufficient, while interpolating in the case of intermediate and extrapolating in the case of lower or higher concentrations of ammonium sulphate. This method is certainly not quite correct, but the error committed is surely smaller than that inherent to the electrometric measuring of the hydrogen-ion concentration.

e. Comparison of the two Methods for Determination of the amount of Surplus Sulphuric Acid in an Egg-albumin solution containing ammonium sulphate.

The determination of the total content of surplus sulphuric acid in an egg-albumin solution containing ammonium sulphate may thus, as just pointed out, be carried out by two different methods. In either case a measuring of the hydrogen-ion concentration is made, and on the basis of this measurement we may then

either find b by means of the main curve table (Fig. 10) and thus the amount of surplus sulphuric acid $(b \cdot e)$ combined with the egg-albumin in 100 cc. experimental liquid, and further by calculation find the amount of surplus acid (t') of the dispersion medium (the sum $b \cdot e + t'$ then represents the total amount of surplus acid), —

or we may by means of the curves of Figures 12, 13, 14 and 15 find q, and thus directly calculate the total content of surplus sulphuric acid $(q \cdot e)$.

It will now be interesting to see, if and to what extent the two methods of determination will give concordant results, and such a comparison will be of special interest whenever we have to deal with solutions having their hydrogen-ion concentration at, or in the neighbourhood of, the isoelectric reaction of the egg-albumin, where the assumptions made in connection with the calculation of the main curve table are not quite correct. We have, therefore, in Table 29 set forth a series of such calculations, made by both procedures, of the total contents of surplus sulphuric acid or ammonia in solutions of two different concen-

TABLE 29. **Total contents of surplus sulphuric acid or ammonia in an ammoniu the main curve table (Fig. 10) a**

Hydrogen-ion-concentration, h, measured	Number, e, of milligr.-equiv. protein-nitrogen in 100 cc. experimental liquid	Concentration of ammonium sulphate, $c = 2.00 \cdot n$						
		Total amount of surplus sulphuric acid (+) or ammonia (÷ in cc. $n/_{1000}$ present in 100 cc. experimental liquid					Difference $t_q \div t_b$ in cc. $n/_{1000}$	Differen per milligr equiv. protei nitroge in cc. $n/_{1}$
		determined by means of the *main curve table* (Fig. 10)			determined by means of Fig. 12 and 14			
$h \cdot 10^6$	e	b	t'	$t_b = b \cdot c + t'$	q	$t_q = q \cdot e$	$t_q \div t_b$	$\frac{t_q \div t}{e}$
10	1	÷ 5.88	+ 7.04	+ 1.16	+ 2.19	+ 2.19	+ 1.03	+ 1.0
15	,,	÷ 0.63	12.56	+ 11.93	—	—	—	—
10	2	÷ 5.88	7.04	÷ 4.72	÷ 0.92	÷ 1.84	2.88	1.4
15	,,	÷ 0.63	12.54	+ 11.28	+ 7.40	+ 14.80	3.52	1.7
10	5	÷ 5.88	7.01	÷ 22.39	÷ 3.64	÷ 18.20	4.19	0.8
15	,,	÷ 0.63	12.51	+ 9.36	+ 2.33	+ 11.65	2.29	0.4
20	,,	+ 3.70	17.60	+ 36.10	+ 7.44	+ 37.20	1.10	0.2
25	,,	+ 7.12	22.53	+ 58.13	—	—	--	—
10	10	÷ 5.88	6.98	÷ 51.82	÷ 4.92	÷ 49.20	2.62	0.2
15	,,	÷ 0.63	12.45	+ 6.15	+ 0.63	+ 6.30	0.15	0.0
20	,,	+ 3.70	17.51	+ 54.51	+ 5.34	+ 53.40	÷ 1.11	÷ 0.1
25	,,	+ 7.12	22.42	+ 93.62	+ 9.25	+ 92.50	÷ 1.12	÷ 0.1
10	15	÷ 5.88	6.94	÷ 81.26	÷ 5.00	÷ 75.00	+ 6.26	+ 0.4
15	,,	÷ 0.63	12.38	+ 2.93	+ 0.48	+ 7.20	4.27	0.2
20	,,	+ 3.70	17.43	+ 72.93	+ 5.06	+ 75.90	2.97	0.2
25	,,	+ 7.12	22.30	+ 129.10	+ 8.93	+ 133.95	4.85	0.3
10	20	÷ 5.88	6.91	÷ 110.69	÷ 5.07	÷ 101.40	9.29	0.4
15	,,	÷ 0.63	12.32	÷ 0.28	+ 0.37	+ 7.40	7.68	0.3
20	,,	+ 3.70	17.34	+ 91.34	+ 4.91	+ 98.20	6.86	0.3
25	,,	+ 7.12	22.19	+ 164.59	+ 8.71	+ 174.20	9.61	0.4

phate containing solution of egg-albumin determined both by means of curves in Fig. 12, 13 and 14.

Concentration of ammonium sulphate, $c = 0.20 \cdot n$

Total amount of surplus sulphuric acid (+) or ammonia (÷) in cc. $n/_{1000}$ present in 100 cc. experimental liquid					Difference $t_q \div t_b$ in cc. $n/_{1000}$	Difference per milligr.-equival. protein nitrogen in cc. $n/_{1000}$
determined by means of the *main curve table* (Fig. 10)			determined by means of Fig. 12 and 13			
b	t'	$t_b = b \cdot e + t'$	q	$t_q = q \cdot e$	$t_q \div t_b$	$\frac{t_q \div t_b}{e}$
÷ 4.95	+ 3.15	÷ 1.80	+ 1.58	+ 1.58	+ 3.38	+ 3.38
÷ 0.42	5.02	+ 4.60	+ 7.78	+ 7.78	3.18	3.18
÷ 4.95	3.14	÷ 6.76	÷ 1.35	÷ 2.70	4.06	2.03
÷ 0.42	5.01	+ 4.17	+ 4.70	+ 9.40	5.23	2.62
÷ 4.95	3.14	÷ 21.61	÷ 3.94	÷ 19.70	1.91	0.38
÷ 0.42	5.00	+ 2.90	+ 1.30	+ 6.50	3.60	0.72
+ 3.13	6.80	+ 22.45	+ 5.44	+ 27.20	4.75	0.95
+ 5.96	8.57	+ 38.37	+ 8.69	+ 43.45	5.08	1.02
÷ 4.95	3.12	÷ 46.38	÷ 4.70	÷ 47.00	÷ 0.62	÷ 0.06
÷ 0.42	4.98	+ 0.78	+ 0.03	+ 0.30	÷ 0.48	÷ 0.05
+ 3.13	6.77	+ 38.07	+ 3.93	+ 39.30	+ 1.23	+ 0.12
+ 5.96	8.54	+ 68.14	+ 7.13	+ 71.30	3.16	0.32
÷ 4.95	3.11	÷ 71.14	÷ 4.76	÷ 71.40	÷ 0.26	÷ 0.02
÷ 0.42	4.96	÷ 1.34	÷ 0.09	÷ 1.35	÷ 0.01	0.00
+ 3.13	6.74	+ 53.69	+ 3.68	+ 55.20	+ 1.51	+ 0.10
+ 5.96	8.50	+ 97.90	+ 6.81	+ 102.15	4.25	0.28
÷ 4.95	3.10	÷ 95.90	÷ 4.81	÷ 96.20	÷ 0.30	÷ 0.02
÷ 0.42	4.93	÷ 3.47	÷ 0.16	÷ 3.20	+ 0.27	+ 0.01
+ 3.13	6.71	+ 69.31	+ 3.57	+ 71.40	2.09	0.10
+ 5.96	8.46	+ 127.66	+ 6.66	+ 133.20	5.54	0.28

trations of ammonium sulphate ($c = 2.0 \cdot n$ and $c = 0.2 \cdot n$), of different concentration of protein ($e = 1, 2, 5, 10, 15$, or 20) and of the hydrogen-ion concentrations 10, 15, 20 and $25 \cdot 10^{\div 6}$.

Table 29 does not require any further explanation if the signification of the quantities b, t', q and t is remembered; t is here designated partly by t_b and partly by t_q, in order to distinguish between the results of the two methods of calculation. We shall only point out the fact that b (if $c = 2.00 \cdot n$) is deduced from the main curve table (Fig. 10) by interpolation between the curves corresponding with $c = 2.817 \cdot n$ and $c = 0.363 \cdot n$, while b (if $c = 0.20 \cdot n$) is deduced in the same manner from the curves corresponding with $c = 0.363 \cdot n$ and $c = 0.062 \cdot n$, because, as mentioned above (see p. 128), the course of the curve corresponding with $c = 1.417 \cdot n$ differs a little from that of the other curves just at the hydrogen-ion concentrations in question. In the same manner we have found q (supposing $c = 2.00 \cdot n$) by interpolation, using the bunches of curves corresponding with $c = 2.79 \cdot n$ (Fig. 14) and $c = 0.36 \cdot n$ (Fig. 12), just as we (if $c = 0.20 \cdot n$) have found q by means of the bunches of curves corresponding with $c = 0.36 \cdot n$ (Fig. 12) and $c = 0.064 \cdot n$ (Fig. 13). t' is calculated in exactly the same way as described in our comments on Table 22 (see p. 123)[1]).

It appears from Table 29 that the differences between the results obtained by the two methods are generally but small. The greatest absolute differences are of course found in the protein rich solutions: but if the difference is calculated per milligram-equivalent protein nitrogen, it is greatest in the case of low concentrations of protein, and the quantity $\frac{t_q \div t_b}{e}$ *stated in the table is always in such cases positive. This fact agrees exactly with what was to be expected,* since at the hydrogen-ion concentrations here in question b deduced from the main curve table (Fig. 10) is only correct when the protein concentrations are comparatively high, but on the contrary is too low at low concentrations of protein (see p. 136). In such cases t_b will, therefore, also be too low, and $\frac{t_q \div t_b}{e}$ will be positive. The table shows that, *if $c = 1$ or $c = 2$, the difference per milligram-equivalent protein nitrogen may*

[1]) We have in Table 29, just as in the preceding tables, constantly stated all the quantities with two decimals, though we know that even the first decimal cannot generally be considered reliable.

amount to 2—3 cc. $^n/_{1000}$: thus the error is here considerable, but for the determination of the total amount of surplus acid it is only of slight consequence, because the protein concentration is low. *Already if e = 5, the differences are smaller, not exceeding 1 cc. $^n/_{1000}$: and with greater protein concentrations the differences per milligram-equivalent protein nitrogen never reach a magnitude of $^1/_2$ cc. $^n/_{1000}$.*

D. The isoelectric Point of Egg-albumin.

In a paper[1]) published a few years ago, L. Michaelis sets up the following thesis: If an easily soluble ampholyte is added to a solution of known hydrogen-ion concentration, the ampholyte will behave as an acid if the hydrogen-ion concentration is smaller, and as a base if the hydrogen-ion concentration is greater than that corresponding with the isoelectric reaction of the ampholyte. In the former case the ampholyte will — like any acid — increase the hydrogen-ion concentration, whereas in the latter case — like any base — it will diminish the concentration. If the original hydrogen-ion concentration of the solution is equal to that corresponding with the isoelectric point of the ampholyte, the addition of the latter will produce no alteration in the hydrogen-ion concentration of the solution. In order to prove the correctness of this "assertion" — to use Michaelis' own mode of expression —, the author sets forth a series of experiments with the ampholytes phenylalanin and glycocoll, the results of which agree tolerably with his theory.

Exactly the same principle is applied by K. A Hasselbalch[2]) in a recently published paper, in which he thoroughly inquires into the well-known fact that blood in normal conditions has a greater hydrogen-ion concentration than that of the corresponding serum. Hasselbalch arrives at the result that this difference between the hydrogen-ion concentration of blood and that of serum disappears little by little as the carbonic acid tension and consequently the hydrogen-ion concentration increase, and at a carbonic acid tension of about 150 mm. the blood and serum (at 38°) will have the same hydrogen-ion concentration corresponding with

[1]) Biochem. Zeitschr. **47**, 251 (1912).
[2]) *Ibid.*, **78**, 129 (1916).

p_H = about 6.8. The cause is attributed by Hasselbalch to the oxyhæmoglobin contained in the blood, the isoelectric point of which — according to L. Michaelis and D. Takahashi[1]) — is situated precisely at p_H = 6.8, and Hasselbalch believes it possible thus to determine the isoelectric point of oxyhæmoglobin and also of other ampholytes with isoelectric reaction within a range of hydrogen-ion concentration limited by the experimental arrangement.

Neither Michaelis nor Hasselbalch gives a direct proof of the correctness of the mentioned rule, the main purport of which may be briefly expressed as follows: If the hydrogen-ion concentration of a solution is not altered by addition of an ampholyte, the hydrogen-ion concentration of the solution must be equal to the isoelectric point of the ampholyte. As the question, however, is of great significance and moreover of special interest in our investigations, we may be allowed to discuss the question more closely after having talked it over with Dr. K. A. Hasselbalch and also with Professor N. Bjerrum.

In a large, mainly compilating paper in "Ergebnisse der Physiologie"[2]), S. P. L. Sörensen has established a series of rules applicable to aqueous solutions of ampholytes; one of these rules is the following: "The amount of an acid or base which is required to give an aqueous solution of an ampholyte the isoelectric reaction, is independent of the concentration of ampholyte, and equal to the amount of acid or base required to give pure water the hydrogen-ion concentration corresponding with the isoelectric reaction of the ampholyte". It is obvious that the thesis of Michaelis-Hasselbalch must therefore be correct in all cases where the rule laid down by Sörensen is found to hold good. As the latter, however, is deduced by means of equations applicable to salt-free solutions of ampholytes, a more general proof of the correctness of the thesis set up by Michaelis-Hasselbalch will be desirable. Bjerrum has drawn our attention to the fact that the simplest way of accomplishing this proof is by implication.

Taking some salt-free or salt-containing solution with a hydrogen-ion concentration exactly equal to the isoelectric reaction

[1]) Biochem. Zeitschr., **29**, 439 (1910).
[2]) Jahrgang, **12**, 503 (1912).

of the ampholyte, RHOH, we are then able to prove that an addition of any amount of this ampholyte will not alter the hydrogen-ion concentration of the solution. Suppose for instance, that the hydrogen-ion concentration is increased by the dissolution of the ampholyte; this means that the ampholyte adds to the solution more hydrogen-ions than hydroxyl-ions; but at the same time more ampholyte-anions, ROH^{-}, than ampholyte-kations, RH^{+}, must then be formed. This is, however, contrary to our assumptions; for if after the dissolution of the ampholyte the hydrogen-ion concentration is really — as was supposed — greater than that corresponding with the isoelectric reaction, the amount of ampholyte-anions must — according to the definition of the term "isoelectric reaction" — be smaller than the amount of ampholyte-kations. In an analogous manner it is possible to prove that also the hydrogen-ion concentration cannot be diminished by the dissolution of the ampholyte, and we are therefore warranted in supposing the thesis of Michaelis-Hasselbalch to be universally applicable.

We intend in this section to use this rule for the determination of the isoelectric reaction of egg-albumin. By means of the measurements mentioned in the preceding sections, we determine what concentration of surplus sulphuric acid, and consequently what hydrogen-ion concentration, a certain ammonium sulphate solution must have, if its hydrogen-ion concentration is not to be altered by addition of any amount of egg-albumin. Any of the above-mentioned series of experiments — with constant concentration of ammonium sulphate and of egg-albumin, but with different hydrogen-ion concentrations in each experiment of the series — may be used for such a determination of the isoelectric point of the egg albumin, if it is possible by means of such a series to give a graphical sketch of the dependence of h (the hydrogen-ion concentration) on q (the amount, in cc. $^{n}/_{1000}$, of surplus sulphuric acid or ammonia per milligram-equivalent of protein nitrogen).

In order to elucidate the method more closely we may state the details of five of the eleven series of experiments set forth in Table 27 (p. 138). The concentration of ammonium sulphate was in all of these series the same, *viz.* about 0.36 · n. while the concentration of egg-albumin varied from one series to another. In the five series here in question e had the values: 1.023,

2.046, 10.52, 16.33 and 42.24, and in Fig. 11 (p. 140) the curves are found which show the dependence of h on q in these five series. By means of these curves — which group together the experimental results and at the same time compensate the experimental errors —, it is now possible to find graphically the values of q corresponding with the values of h in the neighbourhood of the isoelectric point, which is easily estimated to be situated between $10 \cdot 10^{\div 6}$ and $20 \cdot 10^{\div 6}$ (see p. 128). In Table 30 are put together the values of q found graphically — by means of Fig. 11 — for hydrogen-ion concentrations from $10 \cdot 10^{\div 6}$ to $20 \cdot 10^{\div 6}$ in all five series. As e means the number of milligram-equivalents of protein nitrogen in 100 cc. experimental liquid, $q \cdot e$ will represent the total amount, in cc. $^{n}/_{1000}$, of surplus sulphuric acid (+) or ammonia (÷) present in 100 cc. experimental liquid. If now the volume of the disperse phase in 100 cc. experimental liquid is calculated in the way described above (p. 122), the volume of the dispersion medium will be known too. If the last-mentioned volume is designated V_a, and if the whole amount of surplus sulphuric acid or ammonia present is supposed to be distributed exclusively in this volume, the amount of surplus sulphuric acid or ammonia existing in 100 cc. of such a solution of ammonium sulphate will be equal to $\frac{q \cdot e \cdot 100}{V_a}$. This quantity which we have designated t, is also set forth in Table 30 for all five series of experiments. Finally, the second vertical column of Table 30 contains the amounts of sulphuric acid t_o, expressed in cc. $^{n}/_{1000}$, which must be contained in 100 cc. of the protein-free ammonium sulphate solution, if the hydrogen-ion concentration is to be equal to that stated in the first vertical column. t_o is found by calculation of the concentration of surplus acid s by Formula (7) (p. 76):

$$s = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s},$$

using (for $c = 0.36 \cdot n$) the value 4.40 of f_s and $248.6 \cdot 10^{\div 12}$ of $(x_{max})^2$.

If the figures of Table 30 are represented graphically with h as abscissa, t_o and t respectively as ordinates, we shall obtain the curves set forth in Fig. 16. These curves express the dependence of the hydrogen-ion concentration on the amount of surplus sulphuric acid or ammonia present, both in a protein-free

TABLE 30.

Dependence of hydrogen-ion concentration on surplus sulphuric acid (+) or ammonia (÷) at constant concentration of ammonium sulphate ($c = 0.36 \cdot n$) and at different concentration of egg-albumin (e = the number of milligram-equivalents protein nitrogen in 100 cc. experimental liquid).

q denotes the amount in cc. $n/_{1000}$ of surplus sulphuric acid (+) or ammonia (÷) per milligram-equivalent protein nitrogen.

V_a denotes the volume of ammonium sulphate solution present in 100 cc. experimental liquid.

The total amount of surplus sulphuric acid (+) or ammonia (÷) in 100 cc. protein-free ammonium sulphate solution will therefore be $t = \frac{q \cdot e \cdot 100}{V_a}$.

Hydrogen-ion concentration, h.	$e = 0$ (Protein-free solution of ammonium sulph.)	$e = 1.023$ $V_a = 99.91$		$e = 2.046$ $V_a = 99.82$		$e = 10.52$ $V_a = 99.06$		$e = 16.33$ $V_a = 98.54$		$e = 42.24$ $V_a = 96.23$	
$h \cdot 10^6$	t_0	q	t	q	t	q	t	q	t	q	t
10	3.84	0.18	0.18	÷ 2.20	÷ 4.51	÷ 4.95	÷ 52.57	÷ 5.22	÷ 86.50	÷ 5.53	÷ 242.71
11	4.33	1.90	1.95	÷ 0.86	÷ 1.76	÷ 3.86	÷ 40.99	÷ 4.15	÷ 68.77	÷ 4.45	÷ 195.31
12	4.81	3.60	3.69	0.55	1.13	÷ 2.80	÷ 29.74	÷ 3.10	÷ 51.37	÷ 3.42	÷ 150.10
13	5.29	5.20	5.32	1.88	3.85	÷ 1.78	÷ 18.90	÷ 2.10	÷ 34.80	÷ 2.40	÷ 105.34
14	5.76	6.82	6.98	3.10	6.36	÷ 0.78	÷ 8.28	÷ 1.10	÷ 18.23	÷ 1.42	÷ 62.32
15	6.22	8.30	8.50	4.32	8.86	0.22	2.34	÷ 0.18	÷ 2.98	÷ 0.50	÷ 21.95
16	6.69	9.70	9.93	5.50	11.28	1.18	12.53	0.72	11.93	0.40	17.56
17	7.15	11.00	11.26	6.55	13.43	2.10	22.30	1.62	26.84	1.30	57.06
18	7.61	—	—	7.60	15.58	3.00	31.86	2.48	41.09	2.12	93.05
19	8.06	—	—	8.55	17.53	3.85	40.89	3.30	54.68	2.98	130.79
20	8.52	—	—	9.45	19.37	4.65	49.38	4.08	67.61	3.75	164.59

ammonium sulphate solution (the curve $e = 0$) of concentration $0.36 \cdot n$ and in protein-containing solutions of the same ammonium sulphate concentration (the rest of the curves). According to the theory, all these curves ought to intersect at the same point, and this point should correspond with the isoelectric reaction of the egg-albumin. If we consider, for instance, the curves $e = 0$ and

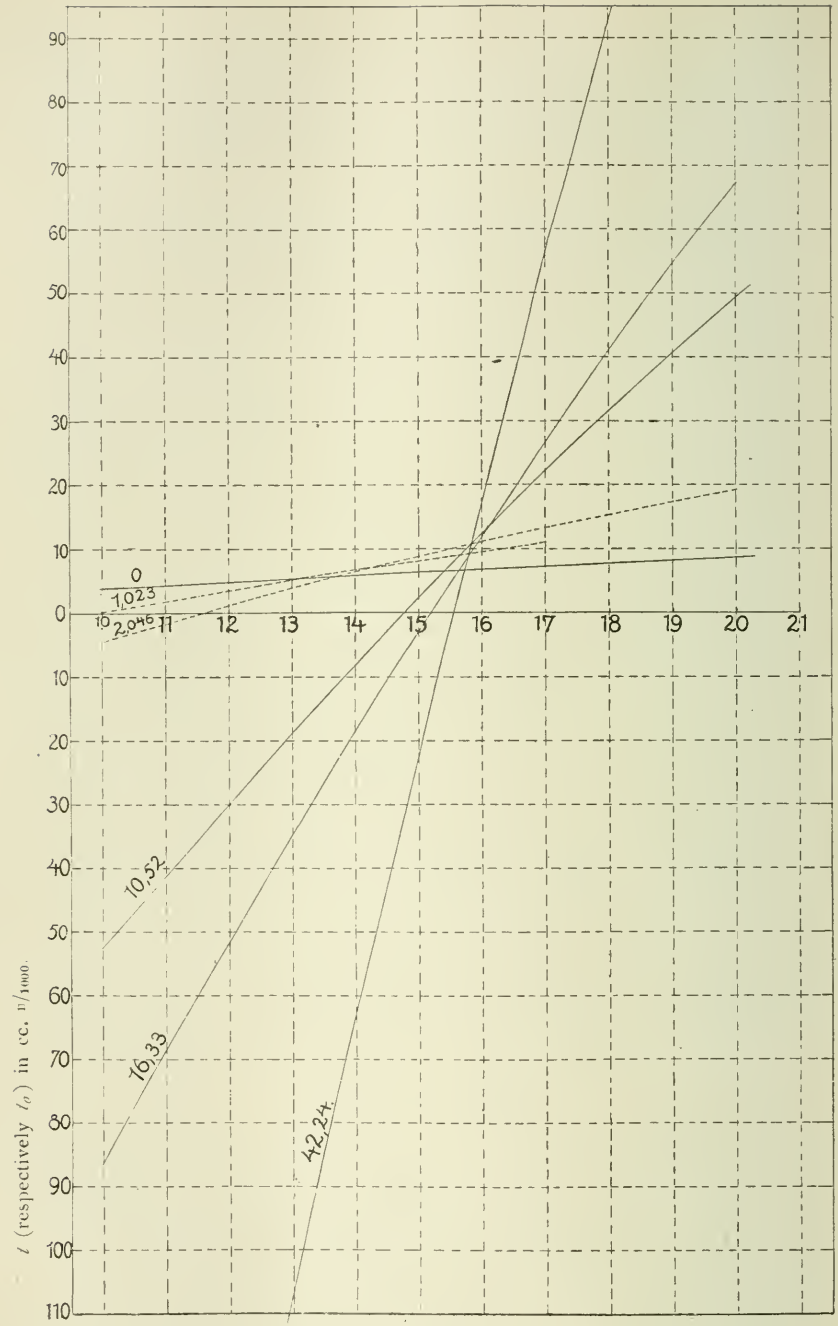
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
10
11
12
13
14
15
16
17
18
19
20
21
0
1,023
2,046
10,52
16,33
42,24
l (respectively l_0) in cc. $^n/_{1000}$.
$k \cdot 10^6$

Fig. 16.

$e = 42.24$, they intersect at a point with the co-ordinates $h = 15.73$ and $t = 6.56$. On account of this point being situated on both curves, the pure ammonium sulphate solution containing in 100 cc. 6.56 cc. $^{n}/_{1000}$ sulphuric acid as well as the protein-containing solution of the same composition must have the same hydrogen-ion concentration: $15.73 \cdot 10^{\div 6}$. Thus this particular ammonium sulphate solution with this particular content of surplus sulphuric acid has not altered its hydrogen-ion concentration on addition of egg-albumin; accordingly, the hydrogen-ion concentration $15.73 \cdot 10^{\div 6}$ must correspond with the iso-electric reaction of the egg-albumin.

In exactly the same manner it is seen, that the points of intersection of the curve $e = 0$ and any of the other curves will give a value of the isoelectric reaction of the egg-albumin, and theoretically all these points ought, of course, as above mentioned, to coincide. Fig. 16 shows, however, that it is not so, and the reason is exclusively attributable to experimental errors, such as are rendered particularly conspicuous on account of the large scale of Fig. 16. If we read off the hydrogen-ion concentrations corresponding with the points of intersection between the ammonium sulphate curve and the other five curves, we get the following values of the isoelectric point h_i:

$e = 1.023$	$h_i = 13.10 \cdot 10^{\div 6}$
$e = 2.046$	$h_i = 13.70$ „
$e = 10.52$	$h_i = 15.42$ „
$e = 16.33$	$h_i = 15.64$ „
$e = 42.24$	$h_i = 15.73$ „

The value of h_i found in the last three series of experiments may be considered as practically identical, the differences being much smaller than the experimental errors. As regards the first two series, they were made on solutions of the sample of egg-albumin designated P_V, which — as mentioned before (see p. 133) — has been found to behave a little differently from the other samples of egg-albumin. Further, as these two experimental series comprehend only comparatively few experiments (three in each series), and as the experimental errors, owing to the slight content of protein, acquire a proportionally great significance, we should not be warranted in attributing to the results obtained from the two series the same value as to the results

from the last three series. We have only stated the results of the first two series for the sake of completeness, and in order to show the limits within which the found values of h_i oscillate; but in the calculation of the mean value of h_i we have left these two values out of consideration, just as we have for the same reason dotted the corresponding curves in Fig. 16.

In exactly the same way as above described we have worked up all the series of experiments suited to such treatment, and the results are set forth in Table 31.

The figures of Table 31 need no further explanation; it is only to be noted that, for the reasons above mentioned, we have parenthesised the results obtained from the series of experiments on the sample of egg-albumin P_I and with low contents of protein. If these series are left out of consideration, it appears from the last vertical column of Table 31 that the isoelectric point of the egg-albumin is situated at $15—16 \cdot 10^{\div 6}$ (the mean value of twenty series of experiments is found to be $15.74 \cdot 10^{\div 6}$). *The situation of the isoelectric point proves to be entirely independent of the concentration of ammonium sulphate, whereas the amount of surplus sulphuric acid naturally increases with increasing concentration of ammonium sulphate. Further, it is seen — with especial clearness from all the experimental series where the concentration of ammonium sulphate is* $0.36 \cdot n$ *— that the situation of the isoelectric point is independent of the concentration of egg-albumin.*

Finally it is seen, that *the isoelectric point is the same in all the examined samples of egg-albumin*, since the differences between the experimental results — at any rate if we omit the experiments stated in parenthesis — are by no means greater than the experimental errors (for the series with $c = 1.41 \cdot n$, see p. 128). As we have examined no less than four different samples of egg-albumin (PIII, PIV and PV used for the measurements executed by S. Palitzsch in 1913 and 1914, and "D. Æ. 6" used by Jenny Hempel in 1915 for the measuring of the series of the main experiment), we think ourselves justified in considering it an established fact that the *isoelectric point of the egg-albumin is situated at a hydrogen-ion concentration of* $15—16 \cdot 10^{\div 6}$.

TABLE 31.

The isoelectric point of egg-albumin.

Kind and designation of egg-albumin	Number of experiments in the series	Concentration of ammonium sulphate c	Number of milligram-equivalents protein nitrogen in 100 cc. experimental liquid e	Amount of surplus sulphuric acid in 100 cc. ammonium sulphate solution at the isoelectric point of the egg-albumin t (in cc. $^{n}/_{1000}$)	*Isoelectric point,* h_i $h_i \cdot 10^6$
P_I-	2	0.064	2.046	(2.65)	(11.95)
Main experiment I	10	,,	10.55	3.50	15.50
,, H	10	,,	31.72	3.50	15.50
P_{III}	4	0.252	9.98	5.58	15.40
P_I-	3	0.360	1.023	(5.33)	(13.10)
,,	3	,,	2.046	(5.62)	(13.70)
P_{II}-	6	,,	8.17	6.59	15.80
P_{III}	3	,,	9.98	6.62	15.86
Main experiment G	10	,,	10.52	6.42	15.42
P_{III}	5	,,	14.97	6.51	15.62
P_{II}-	16	,,	16.33	6.52	15.64
P_I-	3	,,	20.46	6.34	15.25
P_{II}-	11	,,	24.50	6.45	15.48
Main experiment F	10	,,	31.96	6.45	15.48
,, E	10	,,	42.24	6.56	15.73
P_{III}	3	0.501	9.98	7.43	15.77
,,	3	1.003	9.98	9.93	16.08
P_I-	2	1.41	2.046	(10.36)	(14.65)
Main experiment D	9	,,	4.19	11.96	16.50
,, C	10	,,	10.53	12.24	16.83
,, B	10	,,	31.68	12.10	16.67
P_{III}	4	2.004	9.98	13.23	15.63
P_I-	2	2.79	2.046	(13.98)	(14.25)
Main experiment A	10	,,	10.53	15.70	15.60
P_I-	3	,,	20.46	15.07	15.10
,,	3	4.12	2.046	(14.87)	(12.85)

Average: 15.74.

TABLE 32.

Ammonium sulphate solutions.

Contents, S, of ammonium sulphate in g. per 100 g. water;

Specific gravity, d_s, at 18^0, in proportion to water at 4^0;

Volume, V_s, at 18^0, of the weight of the solution which contains 100 g. water;

$$V_s = \frac{100 + S}{d_s};$$

Equivalent concentration, c; $c = \frac{1000 \cdot S}{V_s \cdot 66.08}$.

S	d_s	V_s	c	S	d_s	V_s	c
0	0.99864	100.136	0.00000	31	1.13574	115.342	4.06723
0.2	0.99985	100.215	0.03020	32	1.13907	115.884	4.17880
0.6	1.00221	100.378	0.09046	33	1.14233	116.428	4.28925
1.0	1.00458	100.540	0.15052	34	1.14555	116.974	4.39860
1.5	1.00748	100.746	0.22531	35	1.14872	117.522	4.50686
2	1.01034	100.956	0.29979	36	1.15184	118.072	4.61403
3	1.01595	101.383	0.44780	37	1.15492	118.624	4.72013
4	1.02141	101.820	0.59450	38	1.15794	119.177	4.82521
5	1.02674	102.265	0.73989	39	1.16093	119.732	4.92923
6	1.03197	102.716	0.88397	40	1.16386	120.289	5.03221
7	1.03709	103.173	1.02673	41	1.16675	120.848	5.15978
8	1.04212	103.635	1.16818	42	1.16960	121.409	5.23508
9	1.04704	104.103	1.30829	43	1.17242	121.971	5.33503
10	1.05187	104.576	1.44710	44	1.17518	122.535	5.43397
11	1.05660	105.054	1.58455	45	1.17790	123.100	5.53197
12	1.06125	105.536	1.72070	46	1.18058	123.667	5.62897
13	1.06582	106.022	1.85555	47	1.18323	124.236	5.72500
14	1.07030	106.512	1.98909	48	1.18585	124.806	5.82010
15	1.07470	107.007	2.12131	49	1.18841	125.377	5.91430
16	1.07901	107.506	2.25223	50	1.19095	125.950	6.00754
17	1.08325	108.008	2.38187	51	1.19345	126.524	6.09989
18	1.08743	108.513	2.51024	52	1.19591	127.100	6.19131
19	1.09153	109.021	2.63735	53	1.19835	127.677	6.28186
20	1.09556	109.533	2.76319	54	1.20073	128.255	6.37154
21	1.09952	110.048	2.88777	55	1.20310	128.834	6.46037
22	1.10341	110.566	3.01111	56	1.20543	129.414	6.54835
23	1.10724	111.087	3.13321	57	1.20773	129.996	6.63544
24	1.11100	111.611	3.25409	58	1.20999	130.580	6.72166
25	1.11471	112.137	3.37377	59	1.21221	131.165	6.80705
26	1.11836	112.665	3.49228	60	1.21441	131.751	6.89164
27	1.12194	113.196	3.60959	61	1.21658	132.338	6.97542
28	1.12548	113.729	3.72573	62	1.21872	132.926	7.05841
29	1.12895	114.264	3.84073	63	1.22084	133.515	7.14061
30	1.13238	114.802	3.95455	64	1.22292	134.105	7.22204

SUMMARY.

Section A.

(1) A thorough description is given of the calculation of the hydrogen-ion concentration in an aqueous salt solution containing an excess of the acid or base corresponding with the salt, both in the case of a salt of a strong acid and strong base and in that of a salt of a strong acid and weak base. In the latter case it is necessary in this calculation to take the hydrolysis of the salt into account, when dealing with solutions containing a very small amount of surplus acid, if any.

(2) The formulas found for the calculation of the hydrogen-ion concentration in the case of a salt of the simple composition MAc are applicable also to ammonium sulphate solutions, if the amount of surplus acid is minimal as compared with the amount of ammonium sulphate, the fact being that such an ammonium sulphate solution containing sulphuric acid may be considered as a solution of the acid $H(NH_4SO_4)$ and an excess of the ammonium salt $NH_4(NH_4SO_4)$ of this acid.

(3) Even the purest *preparations of ammonium sulphate in the market do not, generally, contain ammonia and sulphuric acid in equivalent quantities*, but a slight excess of one of the components; this excess amounts, however, seldom to more than $1/50000$ of the whole quantity. The amount of surplus sulphuric acid or ammonia contained in the preparation of ammonium sulphate may be determined by means of measurements of the hydrogen-ion concentration in solutions of the preparation mixed with small and known doses of sulphuric acid.

(4) By means of such measurements of hydrogen-ions it is further possible — by proper use of the formulas mentioned under (1) and (2) — to determine the *acid factor*, f_s, *which is the factor by which the hydrogen-ion concentration of the ammonium sulphate solution has to be multiplied in order to give the concentration of the amount of free sulphuric acid or, to put it more exactly, the amount of acid ammonium sulphate present in the whole solution:* in this amount is included the acid ammonium sulphate formed by the hydrolysis of the ammonium sulphate. f_s is the greater the greater the concentration, c, of the ammonium

sulphate is; the dependence of f_s on c is graphically represented in Fig. 4.

(5) If in an ammonium sulphate solution of known concentration, c, the hydrogen-ion concentration, h, is measured, it is possible to *calculate the concentration, s, of the surplus sulphuric acid in the solution by means of the found value of h.* In this calculation of s the formulæ mentioned under (1) and (2) are used, while taking the hydrolysis of the ammonium sulphate into account, and the value of f_s which enters the calculation being graphically deduced from Fig. 4. A great number of experiments have shown that the error in the value of s very seldom amounts to 1 cc. $^n/_{1000}$ sulphuric acid in 50 cc. experimental liquid, and most frequently it is considerably smaller.

Section B.

(1) The calculation of the hydrogen-ion concentration of acid-containing, salt-free solutions of ampholytes is described in detail. Formulæ are given which permit such a calculation, both if the dissociation of the ampholyte into hydrogen- and ampholyte-anions and into hydroxyl- and ampholyte-kations, respectively, is taken into consideration, and if not. Account is rendered of the nature and magnitude of the error committed in disregarding this dissociation.

(2) In the case of acid-containing solutions of ampholytes which at the same time contain a salt of the acid with a strong base, the calculation of the hydrogen-ion concentration may be executed just as the calculation relating to salt-free solutions, provided that the amount of acid is not minimal.

(3) In acid-containing solutions of ampholytes which further contain a salt of the acid with a weak base, the calculation of the hydrogen-ion concentration is rather complicated, whereas it is comparatively easy to calculate what a concentration of acid such a solution of an ampholyte must have in order to exhibit a definite hydrogen-ion concentration.

(4) In the calculation concerning the salt-containing solutions of ampholytes it has been generally found that it is too complicated to take into account the dissociation of the ampholyte into hydrogen- and ampholyte-anions and into hydroxyl- and ampholyte-kations, respectively. Only in one case we have carried through the complete calculation, and have found a common

expression (Equation 5, p. 105) of *the capacity to combine with acids. that is. the amount of surplus acid combined with one milligram-equivalent ampholyte nitrogen.*

Equation (5) may commonly, without any appreciable error, be reduced to a simpler form (Equation (6), p. 105). It appears from these Equations that *at hydrogen-ion concentrations which are not situated at or in the neighbourhood of the isoelectric point of the ampholyte the capacity to combine with acids*

(a) *is independent of the ampholyte concentration:*

(b) *increases with rising concentration of salt:*

(c) is *positive* at hydrogen-ion concentrations *superior* to that corresponding with the isoelectric reaction of the ampholyte, and

(d) is *negative* — that is, the ampholyte is combined with surplus base — at hydrogen-ion concentrations *inferior* to that corresponding with the isoelectric point of the ampholyte.

At *hydrogen-ion concentrations corresponding with or situated in the neighbourhood of the isoelectric point of the ampholyte, the conditions are not easily discernible: the capacity to combine with acids is in this case not independent of the concentration of the ampholyte, but is the greater the smaller the latter is.*

(5) A series of measurements of hydrogen-ions in acid-containing — salt-free as well as salt-containing — solutions of glycocoll or glycylglycin have shown a perfect accordance between theory and experiment. A calculation of the capacity to combine with acids by means of the measurements of hydrogen-ions has, likewise, given results corresponding exactly with what the common expression of the capacity to combine with acids (mentioned under (4)) demands.

Section C.

(1) *In the case of solutions of egg-albumin the same considerations are applicable as those urged in the examination of the capacity of ampholytes of simple composition to combine with acids.*

(2) In accordance with this, the *capacity of the egg-albumin to combine with acids is independent of the concentration of the egg-albumin at hydrogen-ion concentrations which are somewhat greater or somewhat smaller than that corresponding with the isoelectric point of the egg-albumin* if the concentration of ammonium sulphate is constant. Likewise in accordance with the fore-

going considerations, the capacity to combine with acids or bases, respectively, is at a certain hydrogen-ion concentration *dependent on the concentration of ammonium sulphate, and it is the greater as the latter increases.*

(3) *At hydrogen-ion concentrations at or in the neighbourhood of the isoelectric point of egg-albumin, the capacity of the egg-albumin to combine with acids is to some extent dependent on the concentration of egg-albumin.* and it is (reckoned with sign) the greater the smaller the egg-albumin concentration is. Even this fact may be explained by means of the rules laid down in Section B with regard to the capacity of simple ampholytes to combine with acids.

(4) *Solutions of egg-albumin* prepared by the method described in Paper I always exhibit — the circumstances being supposed to be the same — *the same capacity to combine with acids.* which property can, therefore, be used for characterising the said protein.

(5) By means of the *main curve table* (Fig. 10) on p. 125 it is possible, on the basis of a measurement of the hydrogen-ions in a solution of egg-albumin containing ammonium sulphate and having a known composition, to estimate the *total contents of surplus acid* and with a sufficient approximation to find out the *distribution of the latter between the two phases of the egg-albumin solution.* The calculation of the distribution of the surplus acid is subject to an error which will be the greater the nearer the hydrogen-ion concentration of the solution is to the isoelectric point of the egg-albumin.

(6) The total amount of surplus sulphuric acid contained in an egg-albumin solution with an admixture of ammonium sulphate may also, on the basis of a measurement of hydrogen-ions, be determined in another manner, which is sometimes a little more exact:— namely by means of the bunches of curves represented in Figures 12, 13, 14 and 15. This proceeding is, however, more complicated than that mentioned under (5), and it does not give any information concerning the distribution of the surplus acid between the two phases of the egg-albumin solution.

Section D.

(1) A proof is given of the correctness of the following rule, which was first laid down by L. Michaelis and K. A. Hasselbalch: If the hydrogen-ion concentration of a solution is not

altered by adding an ampholyte, the hydrogen-ion concentration of the solution must be equal to the isoelectric reaction of the ampholyte.

(2) By means of the rule mentioned under (1) and using the measurements described in the preceding sections, the *isoelectric point of egg-albumin is found to be situated at* $15—16 \cdot 10^{-6}$ *(the average of 20 series of experiments gives the value* $15.74 \cdot 10^{-6}$*).*

February 1917.

STUDIES ON PROTEINS.

III. ON THE COMPOSITION AND PROPERTIES OF EGG-ALBUMIN SEPARATED IN CRYSTALLINE FORM BY MEANS OF AMMONIUM SULPHATE.

BY

S. P. L. SÖRENSEN AND MARGRETHE HÖYRUP.

OUR knowledge as to the composition of "crystallised egg-albumin" has hitherto been very deficient, and the reason is to be found in the difficulties offered by a quantitative analytic examination of the crystals of egg-albumin. As it is not possible to prepare the crystals in dry condition proper for analysis, endeavours have been made in other ways to draw conclusions regarding the composition of the crystallised protein.

Franz Hofmeister, who worked out the first method of preparation[1]) of crystallised egg-albumin by means of ammonium sulphate, placed the crystals in alcohol after they had been freed as well as possible from mother liquor by sucking; in the course of a few hours the albumin was in this way transformed into the denaturated insoluble modification, and the ammonium sulphate could then easily be washed away with water. As, in consequence of this treatment, the crystals did not alter their form or show any sign of corrosion, Hofmeister drew the conclusion that the ammonium sulphate present in the crystals originated chiefly, if not entirely, in adherent mother liquor, but formed no integral part of the crystals. For the determination of the elementary composition of the crystallised egg-albumin, Hofmeister therefore used preparations which had been denaturated by alcohol, then washed with water, alcohol and ether, and finally dried at 110° till constant weight.

Several investigators have employed either the same method as Hofmeister or a similar one: they analysed crystallised

[1]) Zeitschr. physiol. Chem. **14**. 165 (1889), **16**. 187 (1891).

egg-albumin which had been previously denaturated either by means of alcohol, or by heating, or by the simultaneous use of both means, and then washed and dried in a proper way. Thus we possess a great number of such analyses executed with the greatest care[1]). The mutual accordance between these analyses made by different experimenters is certainly not quite satisfactory; yet it is only in the case of a single component, namely sulphur, that the discrepancies are considerable. We have not undertaken any analyses of this nature, because it is obvious that in this way it is only possible to get information about the elementary composition of the protein present in the crystallised egg-albumin (and even this will only be possible under the assumption that the elementary composition of the egg-albumin is not altered by the denaturation), — while the analysis gives no information as to the composition of the crystals themselves, and it is especially to this latter question that we have directed our attention. Denaturation and subsequent washing and drying is a so radical treatment that it may result in an essential alteration of the composition, and it will for instance never be possible in this way to get information as to the amount of water that may be contained in the crystals[2]). It is therefore a hasty inference when O. Cohnheim in his book above cited (p. 169) says that K. A. H. Mörner[3]) has proved that the crystallised albumins do not consist of free albumin, but of albumin sulphate. Mörner's investigations relate to egg-albumin which has been crystallised and then denaturated, washed and dried, and from these we dare not — as emphasized above — draw any conclusions concerning the composition of the crystallised albumin. Mörner himself has a clear understanding of this fact, since he writes

[1]) See, for instance, Otto Cohnheim: Chemie der Eiweisskörper, Dritte Auflage, 1911, p. 184.

[2]) On the basis of some elementary analyses of egg-albumin denaturated, washed and then dried at 105°, Fr. N. Schulz (Zeitschr. physiol. Chem. **29**, 89 (1899)) has set forth the theory that the crystallised egg-albumin obtained by an addition of some acid (Krieger's or Hopkins and Pinkus' method) may possibly be a hydrate of the albumin obtained by Hofmeister's method without any addition of acid. Thomas B. Osborne and George F. Campbell (Journ. Americ. Chem. Soc. **22**, 441 (1900)), however, have shown that this is not the case, and, indeed, it could hardly be possible — as mentioned above — that a content of hydrate water should manifest itself in a preparation dried at 105°.

[3]) Zeitschr. physiol. Chem. **34**, 207 (1901).

(*loc. cit.*, p. 250): "Ob auch die Krystalle des Serumalbumins (wie das coagulierte Albumin) aus einem Sulfat bestehen, habe ich noch nicht näher untersucht." Besides, it is only in the examined denaturated preparations of serum-albumin that Mörner finds sulphuric acid, not in the preparations of egg-albumin. He writes (*loc. cit.*, p. 282): »Die Menge der Schwefelsäure ist ganz unbedeutend. Es findet sich keine Berechtigung, von einem "Albuminsulfat" zu sprechen. Trotzdem, dasz das Ovalbumin und das Serumalbumin unter ganz ähnlichen Verhältnissen dargestellt und coagulirt worden waren, verhielten sich diese beiden Eiweisskörper ganz verschieden, indem das Serumalbumin Schwefelsäure aufnahm und so fest zurückhielt, dasz sie nicht durch Waschen entfernt werden konnte, das Ovalbumin aber kein solches Säurebindungsvermögen zeigte." .

F. Gowland Hopkins[1]) has in a way better answering the purpose tried to obtain information concerning the composition of the crystallised egg-albumin. Hopkins found that the egg-albumin crystals, without being denaturated or altering their form or in any way changing their appearance, may be treated with a saturated solution of sodium chloride in acetic acid (containing 1 % acetic acid), and he therefore used such a solution for washing the crystals free from adherent mother liquor. As now it appeared that the crystals of egg-albumin, after being thus carefully washed, did not contain the slightest trace of sulphuric acid, Hopkins drew the conclusion that the crystals probably consist only of egg-albumin, but do not contain ammonium sulphate. He expresses himself very cautiously (*loc. cit*, p. 323): "It may be that such results are not actually conclusive, and that associated ammonium sulphate might be removed from the crystal during treatment, without altering its more obvious optical characters. It is very difficult, however, to see what kind of evidence could be obtained with regard to this question other than the proof that washing may leave the form of the crystal intact and the proteid unaltered, and yet remove all sulphate. Such evidence appears to suggest very strongly that the proteid crystals are formed under the influence of the electrolyte, but not in association with it."

We agree essentially with Hopkins in his considerations;

[1]) Journ. of Physiol. **25**, 320 (1900).

yet it is to be observed that his experiments only deal with the question as to whether the crystals are sulphuric acid free, but leave it undecided whether they contain ammonia or not. This point will be thoroughly discussed in a subsequent section (C, p. 192), giving a detailed account of a series of experiments which we have executed on the principle used by Hopkins.

G. Galeotti[1]) has employed a third method, based upon quite different principles from the two above mentioned methods, in order to decide whether the crystallised egg-albumin consists solely of egg-albumin, or contains ammonium sulphate as well. Galeotti has by pressing between filter paper freed the egg-albumin crystals from the main part of the adherent mother liquor and then determined the weight and the contents of water, ammonium sulphate and egg-albumin of the soft doughy substance. At the same time he determined in weighed samples of the filtrate the amounts present of the same substances: water, ammonium sulphate and egg-albumin. Supposing that all the water found by the analysis of the crystals originated in adherent mother liquor, Galeotti was now able, on the basis of the analysis of the filtrate, to calculate what quantity of mother liquor was present in the doughy crystal mass, and what amounts of egg-albumin and ammonium sulphate corresponded with this amount of mother liquor. It then appeared that the amount of ammonium sulphate originating in the mother liquor was precisely equal to the amount of ammonium sulphate present in the magma; from this result Galeotti concluded that the crystals consisted solely of egg-albumin, and did not contain ammonium sulphate.

The principle employed by Galeotti is the same as that underlying the analytical method described in one of our previous papers[2]). The method is called the "Method of Proportionality", and we have succeeded in applying it to the question here discussed. We have, however, arrived at quite a different result to that of Galeotti, in that — as shown in Section A (p. 169) — by careful and very wide-ranging experiments we have found that the crystals, besides egg-albumin, contain considerable quantities of water. Galeotti's experimental results do not admit of

[1]) Zeitschr. physiol. Chem. **44**, 461 (1905).

[2]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 39 (1916).

such an explanation; he only gives the figures of one single experiment, and from these it appears that, even if the whole content of water of the crystal mixture originate in adherent mother liquor, the corresponding quantity of ammonium sulphate will be a little (0.0005 g.) smaller than the whole amount of ammonium sulphate present in the crystal mass. Accordingly, a content of water in the crystals is out of the question, unless it be supposed — quite gratuitously — that the crystals contain ammonium sulphate at the same time.

We cannot positively say what this discrepancy between Galeotti's and our own experimental results is attributable to; but it may be accounted for on the assumption that during the pressing of the doughy crystal mass in Galeotti's experiment comparatively more water than ammonium sulphate has disappeared; we think, of course, first and foremost of an evaporation of water. If this explanation be correct, the loss of water in Galeotti's experiment must by an odd chance just have been equal to the amount of water contained in the crystals. However, that may be, we believe, on the basis of the experiments described in the following chapters, that we are justified in saying that crystallised egg-albumin — in contradistinction to Galeotti's results — contains considerable quantities of water.

The three methods above described indicate the most important ways in which investigators have attempted to get an insight into the composition of crystallised egg-albumin. A thorough discussion of the different ways in which this question has been dealt with in the rather abundant literature on this matter does not seem to be necessary; we need only add one remark more.

Thomas B. Osborne[1]) has found that, by careful dialysis and subsequent evaporation at 50°, solutions of crystallised egg-albumin give products, the aqueous solution of which show an acid reaction towards litmus and demand measurable quantities of 0.1 · n potassium hydroxide solution to give a neutral reaction towards this indicator, and still greater quantities before neutral reaction towards phenolphtalein occurs. Osborne hence concludes that crystallised egg-albumin is an association of egg-albumin and an acid the nature of which he has not been able to ascertain. That this conclusion is quite destitute of foundation

[1]) Journ. Americ. Chem. Soc. **21**, 477 (1899).

is, however, obvious if we remember the experiments, described in an earlier paper of ours[1]), on the dialysis of egg-albumin solutions containing ammonium sulphate. On account of the hydrolytical processes which take place in the course of the dialysis, it will be impossible from the composition of the matter after the dialysis to draw conclusions concerning the composition of the crystals before dissolution and dialysis have taken place. Besides, the results arrived at by Osborne in the titration of solutions of egg-albumin till neutral reaction towards litmus and phenolphtalein are easily to be understood, if the egg-albumin is considered as an ampholyte of principally acid character, as has been explained in a previous paper[2]).

In what follows we shall discuss in detail the question as to composition of the egg-albumin crystals on the basis of extensive experimental material. In the first three sections we shall deal with the questions as to the possible contents of water (Section A), of surplus sulphuric acid or ammonia (Section B) and of ammonium sulphate (Section C), respectively, while the concluding section (D) contains some few remarks concerning the character of the crystallising process and the form of the crystals.

A. Does crystallised Egg-albumin contain Water?

In the description of the "Method of Proportionality"[3]) we have explained how it is possible by means of analyses of both the "filtrate" and the "precipitate with adherent mother liquor" to get information concerning the composition of the precipitate without adherent mother liquor. It was shown that if the weight of

ammonia nitrogen contained in 100 g. filtrate be designated . a_f
„ „ „ „ „ „ precipitate with adherent mother liquor . . . . a_b
protein nitrogen „ „ „ „ filtrate . . . . . p_f
„ „ „ „ „ „ precipitate with adherent mother liquor. . . . . p_b,

while x, y and z are the factors by which the weight of protein

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 24 (1916).
[2]) *Ibid.*, **12**, 68 (1917).
[3]) *Ibid.*, **12**, 39 (1916).

nitrogen has to be multiplied in order to give the weight of hydrous but ammonium sulphate free egg-albumin (factor: x), the weight of fixed ammonium sulphate (factor: y) and the weight of hydrous egg-albumin containing ammonium sulphate (factor: z), respectively, then it is possible to set up the following equations:

$$z = x + y = r + s \cdot y,$$

where $r = \frac{100\ (a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f}$ and $s = \frac{100\ (p_b \div p_f)}{4.7163\ (a_f \cdot p_b \div a_b \cdot p_f)}$.

Now it has proved — as appears from the following (see p. 178) — to be highly probable that, if we are dealing with crystallised egg-albumin, y will be equal to zero, or in any case quite a minimal quantity as compared with x. We shall therefore for the present leave entirely out of consideration the terms containing y, so that the above equations will be reduced to

$$z = x = r = \frac{100\ (a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f}.$$

We have now to propose to ourselves the problem of *determining the value of r applicable to crystallised egg-albumin* and the *eventual variation of this quantity with the conditions of crystallisation.*

In order to elucidate more closely the method employed, we shall, in regard to one of our experiments, give a thorough description of the experimental arrangement and the results obtained.

To 500 cc. of an egg-albumin solution (recrystallised seven times; 1 cc. solution contained 12.04 mg. of protein nitrogen) 220 cc. saturated solution of ammonium sulphate was added, while carefully shaken, and the resulting mixture received an addition of 10 drops of "inoculating" material. Both the solution of egg-albumin and that of ammonium sulphate had, before being mixed up, a temperature of 19°, and afterwards the mass was left to stand for eleven days in a conical flask closed with a rubber stopper; the flask was placed in a thermostate at 19°, and carefully shaken every day.

The mixture was then filtered (in the thermostate), and in order to avoid evaporation, we used for the filtration one of the common conical filter-flasks with ground-in glass funnel, the upper edge of which was ground and covered with a ground glass plate provided in the middle with an aperture through

which a bent glass tube was placed air-tight, and this glass tube was connected with the side-neck of the filter-flask by means of a rubber tube. On account of the adsorbing power of the filter-paper we did not use the first 20 cc. of the filtrate; they were collected in a graduated cylinder, and it was not until then that the funnel was placed in the filter-flask.

When the filtration in the course of four hours was nearly finished, 3 · 50 cc. of the filtrate were measured and weighed for analyses. From the mass collected on the filter the supernatant mother liquor was removed by means of a pipette, and the precipitate and the residue of the mother liquor were mixed as quickly as possible on the filter by means of a spatula. From the pulpy mixture of precipitate and mother liquor thus obtained, three samples, each about 10 cc., were taken with a pipette without tip, and carefully poured into three weighed 100 cc. graduated flasks; subsequent weighing gave the weight of the precipitate with adherent mother liquor. After dissolving in water and dilution to 100 cc., the three flasks were weighed again, and from each of the three samples were measured, after careful shaking, 4 · 10 cc., which were weighed and used for analyses of the precipitate with adherent mother liquor.

The analyses were executed in the manner previously described[1]): — the protein nitrogen was determined according to Method *b* (*loc. cit.*, p. 50), the ammonia nitrogen in the filtrate after Method *d* (*loc. cit.*, p. 52), and the ammonia nitrogen in the precipitate according to Method *e* (*loc. cit.*, p. 53). The results of the analyses are put together in Table 33 (p. 172—173).

All the figures relating to this experiment are given in the table, which does not need any further explanation, and it is possible by simple calculations to find the quantities a_f, p_f, a_i, p_i, and from these to calculate r.

We have made a large number of experiments of this kind, having varied the conditions of crystallisation in different ways. A summary of these experiments and the found values of r is given in Table 34 (p. 174—175).

The table does not need any further explanation; we shall confine ourselves to drawing attention to the remarks concerning the conditions of crystallisation contained in the third, fourth and

[1]) Comptes-rendus du Laboratoire de Carlsberg. **12**. 48 (1916).

TABL

Filtrate					
		After coagulation, filtration etc.			
Number of flask	Quantity weighed off	the filtrate was made up to 1 l. for determination of ammonia-N; for each distillation 10 cc. was used			the precipitat was used for d termination o protein-N; thi corresp. with c abt. n/14.01 thi sulph. solution
		Weight of 1 l.	Weight of 10 cc.	Ammonia-N corresponding with cc. abt. n/14.01 thiosulphate[1])	
	g.	g.	g.		
4	56.333	1006.68	10.066 10.081 10.080	26.58 26.63 26.65	13.33
11	56.357	1006.70	10.036 10.036 10.026	26.58 26.54 26.53	13.33
12	56.386	1006.81	10.025 10.017 10.030	26.51 26.53 26.54	13.34
Average . . .	56.359	1006.73	10.044	26.565	13.33

100 g. filtrate thus contains 4.6966 g. ammonia-N (a_f)
,, ,, ,, ,, 0.0235 g. protein-N (p_f)

[1] At the titration of ammonia-N the concentration of the thiosulph. was: $0.9941 \cdot n/14.01$

[2] ,, ,, ,, ,, protein-N ,, ,, ,, ,, ,, ,, : $0.9924 \cdot n/14.01$

Using the formula $r = \frac{100\,(a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f}$ for these experimental results, we have found from

Filtrate and Sample I: $r = 7.80_7$
,, ,, ,, II: $r = 7.93_9$
,, ,, ,, III: $r = 7.81_9$

Average . . . . : $r = 7.85_5$

Precipitate with adherent mother liquor

Number of flask	From the 100 cc. 4 · 10 cc. were weighed off Weight g.	Ammonia-N in 10 cc., corresponding with cc. abt. n/14.01 thiosulph. solution[1]	Protein-N in 10 cc., corresponding with cc. abt. n/14.01 thiosulph. solution[2]
Sample I weighed 11.4526 g.; after dissolution and dilution to 100 cc. the weight was 101.354 g.			
13	10.100	2 · 21.53	29.72
14	10.093	2 · 21.59	29.64
15	10.106	2 · 21.62	29.68
17	10.103	2 · 21.51	29.79
Average . . .	10.1005	2 · 21.563	29.71
100 g. of sample I thus contains 3.7563 g. ammonia-N (a_b)			
,, ,, .. ,, ,, 2.5834 g. protein-N (p_b)			
Sample II weighed 11.6101 g.; after dissolution and dilution to 100 cc. the weight was 101.444 g.			
21	10.159	2 · 21.67	31.62
22	10.171	2 · 21.68	31.37
25	10.174	2 · 21.69	31.40
27	10.170	2 · 21.69	31.33
Average . .	10.1685	2 · 21.682	31.43
100 g. of sample II thus contains 3.7042 g. ammonia-N (a_b)			
,, ,, ,, ,, ,, 2.6802 g. protein-N (p_b)			
Sample III weighed 11.0118 g.; after dissolution and dilution to 100 cc. the weight was 101.313 g.			
29	10.100	2 · 20.71	29.29
30	10.115	2 · 20.63	29.31
31	10.066	2 · 20.49	29.24
32	10.129	2 · 20.57	29.49
Average . . .	10.1025	2 · 20.600	29.33
100 g. of sample III thus contains 3.7300 g. ammonia-N (a_b)			
,, ,, ,, ,, ,, 2.6508 g. protein-N (p_b)			

TABLE 34. **Account of the magnitude of the**

$r =$

Number of Experiment	Date	Experimental temperature	Duration of crystallisation in days	Remarks
1	April 1913	5°	2	Variation of experimental temperature and of time of crystallisation.
2	,,	18°	2	
3	,,	11°	12	
4	,,	24°	4	
5	Oct. 1913	4°	3	
6	,,	24°	3	
7	Nov. 1913	18°	10	For the experiment was used a sample of egg-albumin (prepared from rather old eggs) crystallising well, though very slowly.
8	,,	19°	11	"Rapid" These two experiments were treated at the same time and in exactly the same way, only with the difference that the whole amount of ammonium sulphate was added at once in the first experiment, so that the crystallisation took place rapidly; in the second experiment the ammonium sulphate was added little by little, the cryst. thus taking place slowly.
9	,,	,,	,,	"Slow"
10	May 1914	19°	5	In the first of these three experiments the amount of ammonium sulphate was so slight, that only a small fraction of the egg-albumin crystallised out. The filtrate from the first experiment was used for the second, more ammonium sulphate being added; and the filtrate from this was used in the same way for the third experiment. By means of such a fractionated crystallisation an eventual difference in the degree of dispersion might possibly appear.
11	,,	,,	+5 more	
12	,,	,,	+5 more	
13	April 1915	19°	9	While the hydrogen-ion concentration in the first of these experiments was about normal, it was in the second experiment much higher than usual, since the filtrate showed $p_H = 4.417$; $k = 38.28 \cdot 10^{-6}$ (see also p. 186, footnote).
14	,,	,,	,,	

ctor *r*, calculated from the formula

$$r = 100 \cdot \frac{a_f \div a_b}{f_b \div f_f}.$$

100 g. filtrate contained		100 g. precipitate with adherent mother liquor contained				
Ammonia-N in g. (a_f)	Protein-N in g. (f_f)	Sign of sample	Ammonia-N in g. (a_b)	Protein-N in g. (f_b)	r	Average of r
4.7000	0.0228	I	3.8960	2.1871	7.89_0	7.87_9
		II	3.8520	2.3045	7.89_3	
		III	3.9220	2.1267	7.85_4	
4.6870	0.0316	I	4.1050	1.6332	7.73_4	7.81_6
		II	4.0330	1.7983	7.87_8	
		III	4.1400	1.5170	7.83_7	
4.8321	0.0151	I	4.1574	1.7978	7.82_3	7.79_1
		II	4.2147	1.6581	7.76_8	
		III	4.2982	1.4332	7.78_2	
4.8270	0.0161	I	3.9990	2.1870	7.89_1	7.90_1
		II	4.0300	2.0980	7.92_0	
		III	4.0610	2.0240	7.89_3	
4.6460	0.0562	I	4.0910	1.5940	7.73_4	7.83_1
		II	4.0210	1.7480	7.91_6	
		III	4.0580	1.6630	7.84_2	
4.6550	0.0343	I	3.7600	2.4700	7.87_2	7.84_1
		II	3.7620	2.4670	7.86_4	
		III	3.8050	2.3730	7.78_7	
4.7648	0.0377	I	3.6791	2.9043	7.92_4	7.90_2
		II	3.7155	2.8381	7.84_0	
		III	3.6872	2.8763	7.94_3	
4.6966	0.0235	I	3.7563	2.5834	7.80_7	7.85_5
		II	3.7042	2.6802	7.93_9	
		III	3.7300	2.6508	7.81_9	
4.6868	0.0344	I	3.5429	3.0945	7.95_4	7.94_5
		II	3.5465	3.0965	7.92_3	
		III	3.5481	3.0791	7.95_7	
4.0037	0.4036	I	3.0827	3.2368	7.86_2	7.86_1
		II	3.0906	3.2056	7.88_0	
		III	3.0683	3.2886	7.84_2	
4.4191	0.1100	I	3.1788	3.6617	7.83_4	7.83_7
		II	3.2784	3.3710	7.84_7	
		III	3.2062	3.5855	7.82_9	
5.4181	0.0040	I	4.6834	1.7372	7.82_1	7.81_0
		II	4.5686	2.0139	7.79_8	
4.3644	0.1240	I	3.2179	3.4069	7.92_3	7.91_8
		II	3.2120	3.4242	7.92_2	
		III	3.2236	3.3961	7.91_0	
4.3942	0.0551	I	3.4264	2.7959	8.00_0	7.96_8
		II	3.4769	2.6641	7.96_6	
		III	3.3910	2.9184	7.93_8	

Average of the first 12 values . . . **7.86**

fifth columns. It appears from those remarks that we have varied the experimental temperature from 4^0 to 24^0, and the time of crystallisation from 2 to 12 days. We have used both new-laid and older eggs; we have let the crystallisation take place quickly or slowly, we have used fractional crystallisation, and finally we have in a few experiments crystallised at different hydrogen-ion concentration.

In the experiments with fractional crystallisation (Nos. 10, 11 and 12) it might have been expected — from the experiences made by Sven Odén[1]) by fractional precipitation of colloidal solutions of sulphur — that an eventual difference in the degree of dispersion of the egg-albumin would manifest itself; the value of r is, however, as seen from the last column of the table, the same in all three experiments, since the differences are completely within the limits of experimental errors. Much the same may be said of all the experiments under consideration; the last experiment in the table might perhaps be the only exception: in this experiment r is equal to about 7.97, but this is due to the circumstance that the crystallisation was undertaken in a markedly acid liquid. Under such conditions the egg-albumin crystallises — as will be mentioned in a following section (see p. 184) — together with a slight but appreciable and ponderable amount of sulphuric acid, and the factor r will in this case correspond both with the amount of water and that of sulphuric acid; if the value of r is corrected for the content of sulphuric acid (see p. 186, footnote), it will approach to the normal value: 7.86.

On the basis of the experiments stated in Table 34 we now think to be justified in considering it a fact that the *factor r* (if required, after a little correction) *has a constant value, which we have found to be equal to 7.86. and which is independent of the conditions of crystallisation.*

But what is the significance of the factor r? It has been mentioned above (p. 170), that, *under the assumption that crystallised egg-albumin does not contain ammonium sulphate, r is equal to x, and consequently, r represents the factor by which the weight of protein nitrogen has to be multiplied to give the weight of the hydrous egg-albumin. The question is now whether this assumption is correct.*

[1]) Zeitschr. physik. Chem. **78**, 682 (1911); Nova Acta Regiæ Societatis Scientiarum Upsaliensis, Ser. IV, **3** Nr. 4, p. 55 (1912).

In an earlier paper dealing with the Method of Proportionality[1]) we have pointed out that, inasmuch as the common equation:—

$$z = r + s \cdot y$$

contains two unknown quantities, z and y, it is not possible by means of a single experiment, from which only r and s can be calculated, to give a unique solution of the equation; an arbitrarily chosen value of y will give a corresponding value of z. Consequently, no absolute answer to the question can be given on the basis of the above mentioned experiments.

If, however, we consider that the fundamental principle of the Method of Proportionality is based upon a determination of the difference between the amounts of a certain substance (here ammonia nitrogen) in the filtrate and in the precipitate with adherent mother liquor, it is obvious that an eventual content of ammonium sulphate as a component part of the crystals will be of the greater consequence as the ammonium sulphate concentration decreases. In other words, we shall not in two experiments with different concentrations of ammonium sulphate find the same value of r; the value will be smaller as the concentration of ammonium sulphate decreases, and the difference will be of greater significance as the amount of ammonium sulphate combined with the crystals increases.

We shall obtain the same result if we consider the formula of r, which — as before mentioned (*loc. cit.*, p. 43) — in a fairly correct form may be written:

$$r = \frac{100}{p_b}\left(1 \div \frac{a_b}{a_f}\right).$$

If the ammonia nitrogen, a_b, does not originate solely in adherent mother liquor, but if a part of it, a_k, forms a component of the crystals, while the rest, a_m, originates in adherent mother liquor and accordingly under equal conditions (same value of p_b) is a certain fractional part of a_f, — the equation may be written:

$$r = \frac{100}{p_b}\left(1 \div \frac{a_m}{a_f} \div \frac{a_k}{a_f}\right).$$

If now $\frac{a_m}{a_f}$ is constant for a given value of p_b, it is easily

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 42 (1916).

seen that the formula will give a smaller value of r the smaller a_f and the greater a_k is.

Now since the results of our experiments, as noted in Table 34, do not show any variation in the value of r with the value of a_f, we think we are thereby justified in concluding that the *egg-albumin crystals are probably either free from ammonia or contain only quite minimal amounts of ammonium sulphate.* In a subsequent section (C p. 192) we shall try to elucidate this question by means of investigations of a different kind.

As regards the content of water in the crystals, it is of course possible to calculate it by means of the factor $r = x = 7.86$, if we know the factor by which the protein nitrogen has to be multiplied to give the weight of the water-free egg-albumin. In order to determine the magnitude of the latter factor, we have executed a series of drying experiments with a solution of egg-albumin of known composition. The method was as follows:—

In a series of wide weighing-bottles with ground-in glass stoppers, 10 or 20 cc. of an egg-albumin solution were measured and weighed. 100 g. of the egg-albumin solution contained 0.7825 g. protein nitrogen and 0.099 g. ammonium sulphate, but neither surplus sulphuric acid nor ammonia. Some of these samples were dried up without any previous treatment, others were first placed for five hours in a thermostate at 55^0—60^0, which treatment resulted in a partial coagulation, while the rest of the samples before the drying up were allowed to stand 3/4 hour at 93^0—97^0, by which treatment the whole mass coagulated to a thick jelly. The drying up was carried out *in vacuo* over solid hydroxide of potassium at indoor temperature, and was continued for some months, till the monthly loss of weight was smaller than one milligram. The experimental results are set forth in Table 35.

It appears from the first seven vertical columns of the table, which need no further explanation, that the factor sought is very nearly equal to 6.4, since the average of all the determinations is 6.39_2. The previous treatment of the egg-albumin solution does not seem to influence the magnitude of the factor in the slightest degree, since the discrepancies between the results of the separate single experiments may very well originate in experimental errors. The fact that the factor relating to the samples

TABLE 35. **Drying up of Egg-albumin Solutions.**

(100 g. egg-albumin solution contained 0.7825 g. protein nitrogen and 0.099 g. ammonium sulphate).

Number of Experiment	Preliminary treatment	The egg-albumin solution weighed off had		Accordingly, 100 g. egg-albumin solution will contain		Factor Egg-albumin solids divided by Egg-albumin nitrogen	Amount of soluble protein nitrogen contained in the quantity of solids corresponding with 100 g. egg-albumin solution		Remarks
		the weight	the content of solids	Solids	Egg-albumin solids (solids ÷ 0.099 g.)		g.	% of the original amount of soluble protein nitrogen	
		g.	g.	g.	g.				
1	no	20.236	1.0303	5.091	4.992	6.38_0	—	—	Used for crystallisation.
2	,,	20.252	1.0306	5.089	4.990	6.37_7	0.7032	89.87	
3	,,	10.096	0.5147	5.098	4.999	6.38_9	0.6925	88.50	
4	,,	10.101	0.5142	5.091	4.992	6.38_0	—	—	
5	5 hours at 55°—60°	20.268	1.0346	5.105	5.006	6.39_7	—	—	Used for crystallisation.
6	,,	20.264	1.0342	5.104	5.005	6.39_6	0.5863	74.93	
7	,,	10.144	0.5190	5.116	5.017	6.41_2	0.5144	65.74	
8	,,	10.142	0.5184	5.111	5.012	6.40_5	—	—	
9	¾ hour at 93°—97°	20.085	1.0255	5.106	5.007	6.39_9	—	—	Used for crystallisation.
10	,,	20.121	1.0248	5.093	4.994	6.38_2	no	0.00	
11	,,	10.092	0.5146	5.099	5.000	6.39_0	no	0.00	
12	,,	10.095	0.5151	5.103	5.004	6.39_5	—	—	

Average . . . 6.39_2

not previously treated (Nos. 1—4) is a little smaller than those of the others, and that the completely coagulated samples (Nos. 9—12) give a factor somewhat smaller than the partially coagulated (Nos. 5—8) may thus be attributable to accidental circumstances. The difference is in any case so slight that we have not found it necessary, for the sake of the question here at issue, to try to ascertain whether accidental circumstances come into play or not.

We used a number of the dried and weighed samples for determining how much soluble protein nitrogen the dried remainders contained, and an aqueous extract of other samples was used for a crystallising experiment in order hereby to get information as to whether the egg-albumin had suffered any alterations by the previous treatment and subsequent drying up.

From the last vertical column but one in Table 35 it is seen that about 90 % of the protein nitrogen is present in soluble form in samples No. 2 and No. 3; the drying up at indoor temperature without previous treatment has thus caused only a slight denaturation. A far greater part of the egg-albumin is denaturated during the previous treatment at 55^0—60^0 (Nos. 6 and 7), and by the treatment at 93^0—97^0 (Nos. 10 and 11) the denaturation was complete.

The crystallising experiments gave a result agreeing completely with that just described. The dried remainder was carefully treated with about 20 cc. water; after filtration, a saturated solution of ammonium sulphate was added to the filtrate in such quantity that a permanent precipitate was just formed; then we filtered again, and the filtrate was inoculated with a drop of inoculating material and allowed to stand for crystallisation, being well stirred from time to time.

While the solution of the remainder of experiment No. 9, as might be expected, gave no precipitate on addition of ammonium sulphate, the solution corresponding with the experiments Nos. 1 and 5 soon began to crystallise, and the precipitate showed under the microscope the common characteristic appearance: small needles collected in bunches and bundles. After crystallisation during four days, the mixtures were filtered and the filtrates analysed. The filtrates corresponding with Nos. 1 and 5 were found to contain per 100 g. water 0.430 g. and 0.578 g. egg-hydrate, respectively, while by a proceeding described in a

previous paper[1]) it was estimated that pure solutions of egg-albumin by crystallisation under the present conditions (concentrations of ammonium sulphate and of hydrogen-ions) would yield filtrates containing about 0.270 and 0.240 g. egg-hydrate per 100 g. water, respectively. Even if — as shown by a simple calculation — by far the greater part of the egg-albumin has crystallised out both in No. 1 and in No. 5, yet more egg-hydrate than normally will in either case be present in the filtrate, and it is plainly apparent that the crystallisation of No. 1 has been more complete than that of No. 5.

We may just mention that the result of these crystallising experiments might suggest that an alteration into non-crystallisable egg-albumin takes place before the denaturation of the egg-albumin; but our experimental material is too small to warrant sure conclusions; we shall, however, in a later work on the denaturation of egg-albumin, revert to this question.

We think to be justified in summing up the result of these drying experiments in the following way: *The factor by which the weight of protein nitrogen has to be multiplied to give the weight of the egg-albumin dried over solid potassium hydroxide in vacuo at indoor temperature is nearly equal to 6.4, and this does not apply solely to soluble crystallisable egg-albumin, but also to denaturated egg-albumin and eventual middle terms between these two substances.*

The factor found agrees pretty well with analyses made by previous investigators on egg-albumin denaturated and then dried up. Thus by analyses of six samples of egg-albumin carefully purified, then denaturated, and finally dried up at 110°, Thomas B. Osborne and George F. Campbell[2]) find a content of 15.32—15.60 % nitrogen, the average being 15.51 %, which corresponds with the factor 6.45. Quite similar results were obtained by all the other hitherto published analyses on carefully purified material.

If we use the factor 6.40 together with the factor 7.86 found above (p. 176) for crystallised egg-albumin, the water-content of the crystals per g. water-free egg-albumin will be:

$$\frac{7.86 \div 6.40}{6.40} = 0.228 \text{ g.},$$

[1]) Comptes-rendus du Laboratoire de Carlsberg **12**, 21 (1916).

[2]) Journ. Americ. Chem. Soc. **22**, 440 (1900).

while the factor 6.45 in the same way will give 0.219 g. *The crystallised egg-albumin thus contains about 0.22 g. water per g. water-free egg-albumin.*

B. Does crystallised Egg-albumin contain Surplus Sulphuric Acid or Ammonia?

In the preceding paper[1]) we have described how it is possible, by means of a measurement of the hydrogen-ion concentration of an egg-albumin solution containing ammonium sulphate, to determine the contents of surplus sulphuric acid or ammonia, if the composition of the solution is otherwise known. Therefore it would also be a comparatively easy matter to answer the question dealt with in this section, provided that the crystals were present in a state of purity without adherent mother liquor, or provided it were possible to disregard the surplus sulphuric acid or ammonia present in the adherent mother liquor. In this case we should only have to dissolve the crystals in water and determine the contents of ammonia and protein nitrogen, as also the hydrogen-ion concentration of the aqueous solution; from the results obtained we should be able to calculate the contents of surplus sulphuric acid or ammonia. It is certain that such a method gives a fairly correct result, since the amount of surplus sulphuric acid or ammonia of the adherent mother liquor in most cases forms only a small fractional part of the amount present in the crystals; but a perfectly correct result can, of course, only be obtained if we take into consideration both the crystals and the mother liquor. The method will hereby become somewhat more complicated, it is true; nevertheless it will cause no real trouble whatever; it only requires some measurements of hydrogen-ions and analyses of both the filtrate and the precipitate with adherent mother liquor in exactly the same way as mentioned in Section A *à propos* the determination of the factor *r* (see p. 169). In fact, by means of such analyses and measurements of hydrogen-ions, it is possible to determine the contents of surplus sulphuric acid or ammonia in the filtrate as well as in the precipitate with adherent mother liquor. Further, as thoroughly described in the preceding section (see p. 177), we think to be warranted in considering it a fact that all the ammonium sulphate

[1]) Comptes-rendus du Laboratoire de Carlsberg **12**, 134 (1917).

found in the precipitate with adherent mother liquor originates in the adherent mother liquor, and we are thus enabled to calculate the amount of the latter and the amount of surplus sulphuric acid or ammonia present in the same; a simple subtraction will then give the amount of surplus sulphuric acid or ammonia contained in the crystals deprived of their mother liquor. In order to elucidate the method more distinctly, we shall state the details regarding a few experiments.

As the *first example* we take the experiment shown in Table 33 (No. 8 in Table 34):

Analysis of the Filtrate: As 50 cc. weighs 56.359 g. and 100 g. filtrate contains 4.6966 g. ammonia nitrogen (a_f) and 0.0235 g. protein nitrogen (p_f), 100 cc. of the filtrate will weigh 112.718 g. and contain 5.2939 g. ammonia nitrogen ($c = 3.78 \cdot n$)[1]) and 0.0265 g. protein nitrogen (1.89 milligram equivalents = c). By the measurement of hydrogen-ions, h was found to be equal to $11.27 \cdot 10^{\div 6}$.

By means of the curves in Fig. 14[2]), which apply to the concentration of ammonium sulphate: $c = 2.79$, we now find that, if $c = 1.89$ and $h = 11.27 \cdot 10^{\div 6}$, q will be equal to + 2.0, since q is the number of cc. $^n/_{1000}$ surplus sulphuric acid (+) or ammonia (÷) per milligram equivalent of protein nitrogen. From Fig. 15 (*loc. cit.*), the curves of which relate to the concentration of ammonium sulphate $c = 4.12$, it is likewise seen that at this concentration of ammonium sulphate, q will be equal to + 5.0 if $c = 1.89$ and $h = 11.27 \cdot 10^{\div 6}$. Hence, as

$$c = 2.79 \text{ gives } q = + 2.0$$
$$\text{and } c = 4.12 \quad ,, \quad q = + 5.0,$$

an interpolation will give the result that q is + 4.2 if $c = 3.78$, the ammonium sulphate concentration of the filtrate. Consequently the amount of surplus sulphuric acid is $+ 4.2 \cdot 1.89 = + 7.94$ cc. $^n/_{1000}$ in 100 cc. filtrate and + 7.94 : 1.12718 = + 7.04 cc. $^n/_{1000}$ i 100 g. filtrate.

Analysis of the Precipitate with adherent mother liquor ($B + M$). Sample I: As 100 g. ($B - M$) according to the analysis contains 3.7563 g. ammonia nitrogen (a_t) and 2.5834 g. protein nitrogen (p_t), the weighed Sample I (weight 11.4526 g.) and the solution prepared from it (100 cc.) will contain 0.4302 g. ammonia nitrogen ($c = 0.31 \cdot n$) and 0.2959 g. protein nitrogen (21.12 milligram equivalents = c). By the measurement of hydrogen-ions, h was found to be equal to $14.61 \cdot 10^{\div 6}$.

Now, as both the curves in Fig. 12 ($c = 0.363 \cdot n$) and those in

[1]) In calculating the equivalent concentration, c, of the ammonium sulphate, we have here disregarded the volume of the egg-hydrate, as the correction term originating in this volume at the low concentrations of protein dealt with in these experiments is insignificant as compared with the experimental errors.

[2]) Comptes-rendus du Laboratoire de Carlsberg **12**, 143 (1917).

Fig. 13 ($c = 0.062 \cdot n$), if $c = 21.12$ and $h = 14.61 \cdot 10^{\div 6}$, give $q = \div 0.5$, q may be put equal to $\div 0.5$ also for the mean value of c (0.31) here in question. The whole amount of surplus sulphuric acid of Sample I is therefore equal to $\div 0.5 \cdot 21.12 = \div 10.56$ cc. $^n/_{1000}$ (that is 10.56 cc. $^n/_{1000}$ ammonia).

Supposing that the whole amount of ammonia nitrogen (0.4302 g.) arises from ammonium sulphate in the adherent filtrate[1]), the weight of the latter will be $\frac{100 \cdot 0.4302}{4.6966} = 9.1598$ g.[2]), in which 0.0022 g. protein nitrogen (0.16 milligram equivalents) is present, while the amount of surplus sulphuric acid according to the above analysis of the filtrate is $+ 7.04 \cdot \frac{9.1598}{100} = + 0.64$ cc. $^n/_{1000}$.

Thus, as $(B + M)$ in Sample I contains in all $\div 10.56$ cc. $^n/_{1000}$ surplus sulphuric acid, and as M contains $+ 0.64$ cc. $^n/_{1000}$ surplus sulphuric acid, it follows that B must contain $\div 10.56 \div (+ 0.64) = \div 11.20$ cc. $^n/_{1000}$ surplus sulphuric acid or, calculated per milligram equivalent of protein nitrogen, $\frac{\div 11.20}{21.12 \div 0.16} = \div 0.53$ cc.

The result is consequently that *the crystals in this experiment contained 0.53 cc. $^n/_{1000}$ surplus ammonia per milligram equivalent protein nitrogen.*

For the *second example* we choose experiment No. 14 (Table 34), in which the crystallisation took place in a somewhat more acid liquid, and the calculation is for this reason made in a somewhat different way:

Analysis of the Filtrate: 100 cc. of the filtrate weighed 112.013 g., and contained 4.9221 g. ammonia nitrogen ($c = 3.51 \cdot n$) and 0.0617 g. protein nitrogen (4.40 milligram equivalents $= c$). By the measurement of hydrogen-ions, h was found to be $38.28 \cdot 10^{\div 6}$.

From the curves in Fig. 10[3]) it now appears that b — which signifies the amount of surplus sulphuric acid, expressed in cc. $^n/_{1000}$, fixed by the egg-hydrate per milligram equivalent protein nitrogen — will, if $h = 38.28 \cdot 10^{\div 6}$, vary in the following manner together with the concentration of ammonium sulphate:

$c = 0.062$; $b = + 11.30$
$,, = 0.363$; $,, = + 13.00$
$,, = 1.417$; $,, = + 13.45$
$,, = 2.817$; $,, = + 14.55$

[1]) In this calculation it is of course permissible to disregard the slight amount of ammonia which — as the final result shows — is contained in the crystals, the corresponding amount of nitrogen forming only a minimal part of the whole amount of ammonia nitrogen present (in this experiment, for instance, only about $^1/_{2700}$ of the whole quantity).

[2]) Thus the weight of the crystals is $11.4526 \div 9.1598 = 2.2928$ g., and as the amount of protein nitrogen of the crystals is $0.2959 \div 0.0022 = 0.2937$ g., the protein factor, r, will be $\frac{2.2928}{0.2937} = 7.807$ (compare Table 33).

[3]) Comptes-rendus du Laboratoire de Carlsberg **12**, 125 (1917).

By means of these values we obtain by graphical extrapolation, if $c = 3.51$: $b = + 15.00$[1]).

The whole amount of surplus sulphuric acid combined with the egg-hydrate of 100 cc. filtrate is thus $+ 15.00 \cdot 4.40 = + 66.0$ cc. $^{n}/_{1000}$.

Further, the concentration, s, of surplus sulphuric acid in the dispersion medium may be calculated from the formula[2]):

$$s = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s},$$

which calculation gives $s = 486 \cdot 10^{\div 6}$. From this we find that the amount, in 100 cc. filtrate, of surplus sulphuric acid not combined with the egg-hydrate is 48.6 cc. $^{n}/_{1000}$, leaving here, as above, the volume of the egg-hydrate out of consideration (see p. 183, note).

The whole amount of surplus sulphuric acid in 100 cc. filtrate is thus: $+ 66.0 + 48.6 = + 114.6$ cc. $^{n}/_{1000}$, and in 100 g. filtrate $+ 114.6 : 1.12013 = + 102.31$ cc.

Analysis of the Precipitate with adherent mother liquor ($B + M$), Sample I:

As 100 g. ($B + M$) according to the analysis contain 3.4264 g. ammonia nitrogen (a) and 2.7959 g. protein nitrogen (p_s), the weighed sample (weight: 6.5141 g.) and the solution (100 cc.) prepared from it will contain 0.2232 g. ammonia nitrogen ($c = 0.159 \cdot$ n) and 0.1821 g. protein nitrogen (13.00 milligram equivalents $= e$). By the measurement of hydrogen-ions, h was found to be $40.36 \cdot 10^{\div 6}$.

From the curves in Fig. 10 (*loc. cit.*) it now appears that, if $h = 40.36 \cdot 10^{\div 6}$, and if

$c = 0.062$, b will $= + 12.02$
$,, = 0.363$, $,,$ $,,$ $= + 13.85$
$,, = 1.417$, $,,$ $,,$ $= + 14.35$
$,, = 2.817$, $,,$ $,,$ $= + 15.45$

By means of these values we obtain by graphical interpolation, if $c = 0.159$: $b = + 13.38$.

The whole amount of surplus sulphuric acid combined with the egg-hydrate of the 100 cc. solution of Sample I will thus be $+ 13.38 \cdot 13.00 = + 173.94$ cc. $^{n}/_{1000}$.

Further, it is possible, by means of the formula

$$s = h \cdot f_s \div \frac{(x_{max})^2}{h \cdot f_s},$$

to calculate that the amount of surplus sulphuric acid not combined

[1]) This value of b cannot of course be particularly exact, since it was found by an extrapolation; and, hence, the content of surplus acid of the filtrate is not determined with particular exactness either. The fact is, however, but of insignificant importance because — as appears from what follows (see p. 186) — the amount of surplus sulphuric acid originating in the adherent mother liquor forms but about $^{1}/_{86}$ of the whole amount of surplus acid existing in the precipitate with adherent mother liquor.

[2]) Comptes-rendus du Laboratoire de Carlsberg **12**, 76 and 123 (1917).

with the egg-hydrate but present in the 100 cc. solution of Sample I, is equal to + 12.85 cc. $^{n}/_{1000}$.

Finally, we obtain, in the same way and under the same assumption as in the first example, that the weight of the mother liquor adherent to the crystals is $\frac{100 \cdot 0.2232}{4.3942} = 5.0794$ g.[1]), in which 0.0028 g. protein nitrogen (0.20 milligram equivalents) is present, while the amount of surplus sulphuric acid according to the above analysis comes to $+ 102.31 \cdot \frac{5.0794}{100} = + 5.20$ cc. $^{n}/_{1000}$.

Consequently, as $(B + M)$ in Sample I contains, in all, + 173.94 + 12.85 = + 186.79 cc. $^{n}/_{1000}$ surplus sulphuric acid, and as M contains + 5.20 cc., B will contain + 186.79 ÷ 5.20 = + 181.59 cc. $^{n}/_{1000}$ surplus sulphuric acid, or, per milligram equivalent protein nitrogen: $\frac{+ 181.59}{13.00 \div 0.20} = + 14.19$ cc.

Thus the result is that *the crystals in this experiment contain 14.19 cc. $^{n}/_{1000}$ surplus sulphuric acid per milligram equivalent protein nitrogen.*

By means of one of the two methods used in the two examples thoroughly described above, we have calculated the amount of surplus sulphuric acid or ammonia present in the crystals in all the experiments noted in Table 34, and, further, in a few other appropriate experiments. In these latter, the analytical method was, however, not sufficiently exact to admit of our using the analysis results for the calculation of the factor r. The results of all the calculations are set forth in Table 36.

The first 14 experiment numbers answer to the corresponding experiment numbers of Table 34. The second, third and fourth columns of the table give information as to the composition and hydrogen-ion concentration of the filtrate, while the last four columns of the table contain the calculated contents of surplus sulphuric acid or ammonia of the crystals. We have represented these results graphically in Fig. 17, using the hydrogen-

[1]) The weight of the crystals will then be 6.5141 ÷ 5.0794 = 1.4347 g., and, as the amount of protein nitrogen in the crystals is 0.1821 ÷ 0.0028 = 0.1793 g., the factor r will be $\frac{1.4347}{0.1793} = 8.00_2$ (compare Table 34). This value of r corresponds, however, not solely with the amount of water in the crystals, but also comprises the content of surplus sulphuric acid. As the latter (see above) amounts to 14.19 cc. $^{n}/_{1000}$ (= 0.696 mg. sulphuric acid) per milligram equivalent protein nitrogen (= 14.01 mg. nitrogen), the corresponding factor will be $\frac{0.696}{14.01} = 0.050$; the corrected value of r will therefore be $8.00_2 \div 0.05 = 7.95_2$.

TABLE 36. **Amount of Surplus Sulphuric Acid or Ammonia in the Crystals.**

Number of Experiment	Filtrate			The crystals contained m cc. $n/_{1000}$ surplus sulphuric acid (+) or ammonia (÷) per milligram equivalent protein nitrogen			
				calculated from the analyses of			Average of
	Contents of protein nitrogen in milligram equivalents, e, per 100 cc. filtrate	Equivalent concentration of ammonium sulphate, c	Hydrogen-ion concentration, h	Sample I	Sample II	Sample III	m
	e	c	$h \cdot 10^6$	m	m	m	
1	18.32	3.78	15.13	+ 0.44	+ 1.20	+ 1.11	+ 0.92
2	2.54	3.77	11.02	÷ 1.44	÷ 1.08	÷ 1.01	÷ 1.18
3	1.22	3.90	11.13	÷ 1.63	÷ 1.54	÷ 1.59	÷ 1.59
4	1.30	3.90	12.06	÷ 1.34	÷ 1.54	÷ 1.46	÷ 1.45
5	4.52	3.74	11.37	÷ 0.51	÷ 0.61	÷ 0.72	÷ 0.61
6	2.74	3.73	12.15	÷ 1.00	÷ 1.22	÷ 1.39	÷ 1.20
7	3.03	3.83	12.46	÷ 0.78	÷ 0.54	÷ 0.79	÷ 0.70
8	1.89	3.78	11.27	÷ 0.53	+ 0.15	÷ 0.43	÷ 0.27
9	2.76	3.76	12.04	÷ 0.03	÷ 0.13	÷ 0.23	÷ 0.13
10	32.47	3.22	10.00	÷ 4.76	÷ 4.75	÷ 4.76	÷ 4.76
11	8.77	3.52	9.73	÷ 6.53	÷ 6.56	÷ 6.51	÷ 6.53
12	0.33	4.43	8.29	÷10.22	÷10.36		÷10.29
13	9.91	3.49	10.57	÷ 2.78	÷ 2.79	÷ 2.78	÷ 2.78
14	4.40	3.51	38.28	+14.19	+14.25	+14.14	+14.19
15	1.18	3.89	11.85	÷ 1.53	÷ 1.44	÷ 1.77	÷ 1.58
16	1.63	3.88	11.45	÷ 1.69	÷ 0.96	÷ 1.25	÷ 1.30
17	4.21	3.48	27.29	+ 8.63	+ 8.59	+ 8.59	+ 8.60
18	6.10	3.49	17.34	+ 2.66	+ 2.68	+ 2.63	+ 2.66

ion concentration, h, as abscissa and the value of m (the last column of Table 36) as ordinate. Through the points thus determined we have drawn a curve which represents the dependence between the hydrogen-ion concentration of the mother liquor (the filtrate) and the contents of surplus sulphuric acid or ammonia of the crystals separated. It is highly probable that other factors than the hydrogen-ion concentration of the filtrate — *c. g.* the ammonium sulphate concentration of the filtrate — are also of

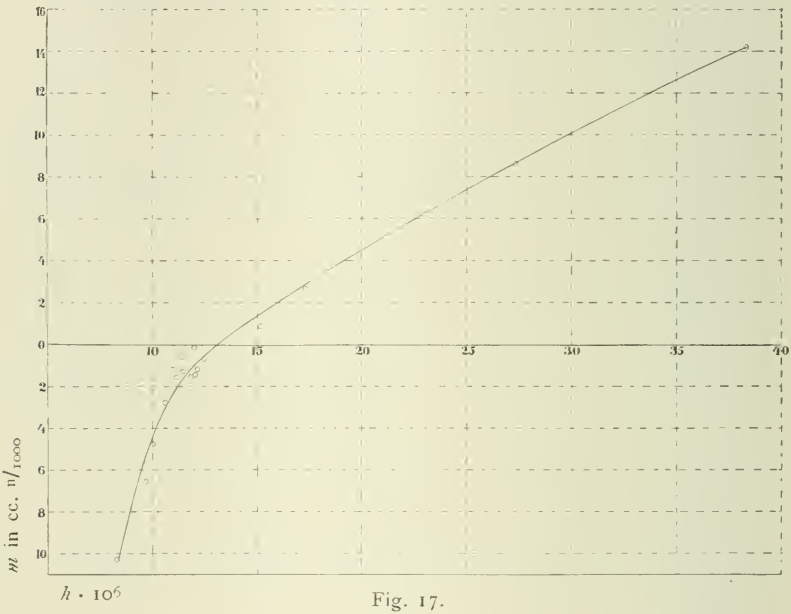

Fig. 17.

consequence with respect to the facts here considered; but from the experimental results it appears clearly that the hydrogen-ion concentration is by far the most important factor, and therefore we have no hesitation in representing all the results by a single curve.

It is seen from Fig. 17, that *only in the case where the mother liquor has a hydrogen-ion concentration of about* $13 \cdot 10^{\div 6}$, *will the egg-albumin crystals separated contain neither surplus sulphuric acid nor ammonia. At higher hydrogen-ion concentrations than about* $13 \cdot 10^{\div 6}$ *the crystals will contain sulphuric acid, below this limit ammonia, and the amount of either substance will increase when the hydrogen-ion concentration increases or decreases, respectively.*

If we compare the curve in Fig. 17 with those in Fig. 10, we observe at once that in both cases the form is very much

the same. As now the curves in Fig. 10 represent the dependence between the hydrogen-ion concentration and the amount of surplus sulphuric acid or ammonia combined with the egg-hydrate present in the solution, while the curve in Fig. 17 shows the dependence between the hydrogen-ion concentration and the amount of surplus sulphuric acid or ammonia combined with the egg-albumin crystallised out, it is an obvious conclusion that the crystallisation merely amounts to a separation in solid condition of the disperse phase of the same composition, or at any rate, with the same content of surplus sulphuric acid or ammonia as that of the disperse phase in the solution. On the whole, this conclusion is correct; yet a closer comparison of the curves in question will show that the state of things is not quite so simple as supposed.

In Table 37 we have put together the values both of m (read from Fig. 17) and of b (partly direct read from Fig. 10, curve IV, corresponding with $c = 2.817 \cdot n$, and partly, for $c = 3.7—3.8 \cdot n$, estimated by graphical extrapolation by means of Fig. 10) corresponding with a series of different values of h.

TABLE 37.

Hydrogen-ion concentration of the mother liquor or the solution	Amount, in cc. $n/_{1000}$, of surplus sulphuric acid (+) or ammonia (÷) per milligram equivalent protein nitrogen.		
	In the *crystals*	In the *disperse phase* of an egg-albumin solution containing ammonium sulphate	
	m	b	b
$h \cdot 10^6$	(Fig. 17)	(Fig. 10, Curve IV; $c = 2.817$)	(Fig. 10, extrapolation; $c = 3.7$-3.8)
10	÷ 4.5	÷ 6.0	÷ 6.3
13.1	0	÷ 2.6	÷ 2.7
15	+ 1.4	÷ 0.6	÷ 0.65
15.6	+ 1.8	0	0
20	+ 4.5	+ 3.8	+ 4.0
25	+ 7.4	+ 7.5	+ 7.95
30	+10.1	+10.6	+11.1
35	+12.6	+13.1	+13.7

As the mean value of the ammonium sulphate concentration, c, of the mother liquor in the crystallisations set forth in Table 36 is $3.7—3.8 \cdot n$, it will be of special interest to compare the values

of b (Table 37, last vertical column) corresponding to this concentration of ammonium sulphate with the corresponding values of m (Table 37, second column). In such a comparison we must, however, remember that the values of b, reckoned with sign, — as proved in the preceding section[1]) — are always too low, and that the error is the greater the more b approaches to zero. Therefore it is necessary to add a correction to the figures stated in the last column of Table 37; this correction being always positive, and greatest in the case of those values of b which are nearly equal to 0. This agrees perfectly with the fact that the egg-albumin, as any ampholyte, at isoelectric reaction is combined with some acid[2]), so that the corrected value of b for $h = 15 - 16 \cdot 10^{\div 6}$ must be positive. If such a correction of b is introduced in the last column of Table 37, it will be seen that the zero is displaced upwards in the series, so that it will be situated at a similar hydrogen-ion concentration to that for the zero of m, while the numerical values of b on both sides of zero are higher than the corresponding values of m.

We are now enabled to give the above mentioned rule a somewhat more extensive form: *At a hydrogen-ion concentration of about* $13 \cdot 10^{\div 6}$ *the egg-hydrate in the solution before crystallisation will neither contain surplus sulphuric acid nor ammonia, and the same will apply to the crystals separated at this hydrogen-ion concentration. The case is somewhat different if the hydrogen-ion concentration be considerably higher or lower than about* $13 \cdot 10^{\div 6}$*:— the crystals separated at higher hydrogen-ion concentrations contain a somewhat smaller amount of surplus sulphuric acid than does the egg-hydrate before crystallisation, while the crystals separated at lower hydrogen-ion concentrations contain a somewhat smaller amount of surplus ammonia than that contained in the egg-hydrate before crystallisation.*

On the whole, however, we must reckon with the circumstance that the egg-hydrate crystallises together with by far the greater part of the surplus sulphuric acid or ammonia which it contains before the crystallisation[3]), and this is the reason why

[1]) Comptes-rendus du Laboratoire de Carlsberg **12**, 135 (1917).

[2]) *loc. cit.* p. 106.

[3]) In perfect accordance herewith we also find that the hydrogen-ion concentration is only altered in a slight degree during crystallisation. In order to

Hopkins and Pinkus's method, adopted by us[1]) for the preparation of crystallised egg-albumin, gives products containing, even after repeated recrystallisations, varying amounts of surplus sulphuric acid or ammonia. The fact is that the dose of sulphuric acid to be added in the first crystallisation is largely a matter of estimate. Hence it follows that the hydrogen-ion concentration, and, consequently, also the amount of surplus sulphuric acid or ammonia combined with the egg-hydrate, will not always be the same in the first crystallisation of the egg-hydrate. As now crystals of egg-hydrate containing for instance an abundant amount of surplus sulphuric acid will give a solution with high hydrogen-ion concentration, from which the egg-hydrate by recrystallisation is again separated with an abundant content of surplus sulphuric acid, it is obvious that even repeated recrystallisations are only partly able to compensate the differences originating in the circumstance that the first crystallisation does not always take place under equal conditions.

The state of things may be rendered more apparent by a few very simple experiments:

In the experiments Nos. 13, 14, 17 and 18 (Table 34) the remainders of precipitates left when the samples had been taken were washed with a solution of ammonium sulphate, the concentration of

settle this question, we have made a series of experiments, in each of which an egg-albumin solution was mixed with a proper quantity of sulphuric acid or ammonia and further with an ammonium sulphate solution till permanent turbidity. Then we filtered, and to the clear filtrate we added a drop of inoculating material, upon which we placed the solution in the electrode vessel and measured the hydrogen-ion concentration, both at once and several times during crystallisation. The following figures may serve as examples of of the results obtained:

$h \cdot 10^6$	
at once	after abundant crystallisation
43.7	43.3
28.2	27.7
19.9	18.3
16.2	14.4
8.9	6.9
4.0	2.9

These figures show that the hydrogen-ion concentration decreases a little in the course of crystallisation, and this alteration is — as might be expected from the above statements — of comparatively greatest significance at low hydrogen-ion concentrations.

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 12 (1916).

which was adjusted as usual[1]), and which contained neither surplus sulphuric acid nor ammonia. Then the precipitates were dissolved separately in water, and from the solutions the egg-hydrate was precipitated anew by the addition of an ammonium sulphate solution. After being left to stand for some days, the mixtures were filtered, the precipitates washed and dissolved, and the crystallisation repeated a third time. In the mother liquor thus obtained, the measurement of the hydrogen-ion concentration gave the undermentioned results, which for the sake of comparison are noted together with the hydrogen-ion concentration of the filtrates of the first crystallisation (see Table 34).

	$h \cdot 10 \div 6$	
	First mother liquor	Third mother liquor
No. 13	10.57	10.38
„ 18	17.34	15.71
„ 17	27.29	24.16
„ 14	38.28	31.77

In the experiments where low hydrogen-ion concentrations were used it is thus only possible to observe a minimal, if any, alteration from the first to the third mother liquor, whereas the experiments with the high hydrogen-ion concentrations show a pronounced decrease of the latter from first to third crystallisation. On the other hand, it is plainly seen from our figures that the crystals from experiment No. 14 must be recrystallised several times if we are to succeed in removing the great content of surplus sulphuric acid in this way.

C. Does crystallised Egg-albumin contain Ammonium Sulphate?

In the introduction it has been already mentioned (see p. 166) that F. Gowland Hopkins[2]) used a saturated sodium chloride solution containing 1 % acetic acid as washing liquid in order to free the egg-albumin crystals from adherent mother liquor, and by such washing repeated several times he succeeded in removing every trace of sulphuric acid, without altering the appearance of the crystals. Hopkins thinks to be justified in drawing from this experiment the conclusion that the crystals probably consist solely of egg-albumin, and do not contain ammonium sulphate. We shall in the present section mention a series of similar experiments made by us in order further to elucidate this question

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 13 (1916).

[2]) Journ. of Physiol. **25**, 320 (1900).

As experimental material we used egg-albumin crystals prepared from diluted solutions of completely purified egg-albumin by slow crystallisation in the course of 3—4 weeks, a small amount of a saturated solution of ammonium sulphate being added every day and the liquid being well stirred. By this slow crystallisation we obtained crystals so large and well-formed that an alteration of form or appearance produced by the washing with sodium chloride solution would be easily perceptible under the microscope.

For the different experiments we used materials which had been crystallised at different hydrogen-ion concentrations (varying between $8 \cdot 10 \div 6$ and $28 \cdot 10 \div 6$). As shown in the preceding section, the materials thus procured were therefore known — setting aside the question as to whether they contained ammonium sulphate or not — to contain surplus ammonia if they had been crystallised at low hydrogen-ion concentrations, and surplus sulphuric acid if crystallised at high hydrogen-ion concentrations, while only the samples crystallised at about $13 \cdot 10 \div 6$ contained neither surplus sulphuric acid nor ammonia.

If now a sample of egg-albumin crystallised without surplus sulphuric acid or ammonia were washed by Hopkins' method with a saturated sodium chloride solution containing 1 % acetic acid and then turned out to give a product of essentially the same appearance after as before the washing, and containing neither sulphuric acid nor ammonia, we could from this result only draw the conclusion already drawn by Hopkins, *viz.* that probably only the mother liquor, but not any of the components of the crystals, had been washed away.

As stated by Hopkins himself, the above explanation, however plausible it may appear, cannot be taken as direct proof. We have thought to obtain conclusive evidence by washing out samples containing either surplus sulphuric acid or ammonia at the same time as the washing of material without surplus sulphuric acid or ammonia. In fact, if Hopkins' supposition were correct, and if accordingly it were possible to remove all the mother liquor without at the same time altering the composition of the crystals, such samples would after washing still contain the same amounts of surplus sulphuric acid or ammonia as before the washing.

The experiments made by us have now given the result that all the washed samples behaved essentially alike, whether the

material used did or did not contain surplus sulphuric acid or ammonia before the washing. The results of our experiments must thus be interpreted as showing that it is not possible from such washing experiments to draw any conclusions as to whether the crystals contain ammonium sulphate or not. For further elucidation of the question we shall render an account of a few of our experiments, from which it will be seen, among other things, with what quantities we worked.

Experiment No. 8. To a diluted solution of egg-albumin recrystallised six times, a little dose of diluted sulphuric acid was added, and then saturated ammonium sulphate solution — in daily portions of 5—10 cc. The egg-albumin thus crystallised in the course of four weeks at indoor temperature, and was filtered[1]); then the precipitate was removed from the filter, placed in a spacious dish and covered with 20—30 times its own volume of a saturated solution of sodium chloride containing 1 % acetic acid. The precipitate was mixed up with this solution as quickly and completely as possible; then it was filtered off and washed eight times with the solution of sodium chloride. In the last washing liquid it was not possible to detect any trace of sulphate by adding barium chloride. The precipitate was again removed from the filter, mixed up with an abundant amount of sodium chloride solution, filtered off, and washed three times, and then the precipitate was once more mixed up with the sodium chloride solution, filtered off, and washed another three times.

The precipitate thus washed was treated with water, by which treatment a fractional part of it was dissolved, while another part remained undissolved, being evidently denaturated, or having become so in the course of the dissolving process. The solution, together with the suspended denaturated precipitate, was made up to 500 cc., of which $2 \cdot 100$ cc. were used for determination of sulphuric acid, and $2 \cdot 100$ cc. for determination of ammonia, while the remainder was used for determination of protein nitrogen and chloride. Our intention in the latter determination was to calculate what quantity of the sodium chloride solution employed for the washing was present, as the sodium chloride solution, though free from sulphuric acid, contained a minimal but known amount of ammonia, which was to be taken into calculation in the determination of the amount of ammonia present in the $2 \cdot 100$ cc. solution with suspended precipitate.

The *determination of protein nitrogen* gave the result that 100 cc.

[1]) An analysis of the filtrate showed that 100 cc. filtrate contained 67.3 mg. protein nitrogen and 4.830 g. ammonia nitrogen, and further — a point of special interest in the case before us — that the hydrogen-ion concentration was $24 \cdot 10 \div 6$, corresponding with $p_H = 4.62$. The egg-albumin crystallised at this hydrogen-ion concentration is, as appears from Fig. 17 (see p. 188), combined with about 6.8 cc. $n/_{1000}$ surplus sulphuric acid per milligram equivalent of protein nitrogen.

solution with suspended precipitate contained 841 mg., or about 60 milligram equivalents, of protein nitrogen.

The *determination of sulphuric acid* was accomplished by a method described in a previous paper[1]), and gave the result that *no sulphuric acid could be detected at all in 100 cc. solution with suspended precipitate.* As it is possible by this test to detect an amount of sulphuric acid of 3 cc. $^{n}/_{1000}$ or more (*loc. cit.* p. 33), and as further the amount of egg-albumin used for the test, which corresponds with 60 milligram equivalents of protein nitrogen, before washing with the sodium chloride solution[2]), has contained $60 \cdot 6.8 = 408$ cc. $^{n}/_{1000}$ surplus sulphuric acid, there can be no doubt that the crystals in this case have lost sulphuric acid in the washing process.

The *determination of ammonia* was made by the procedure described in a previous paper[3]); method *g* was used, and the amount of ammonia determined after Nessler's method. 100 cc. of solution with suspended precipitate was found to contain 0.15 mg. ammonia nitrogen, and as the present solution of sodium chloride corresponded with an amount of ammonia nitrogen of 0.02 mg., *0.13 mg. ammonia nitrogen was found for 60 milligram equivalents of protein nitrogen, or about 0.0022 mg. per milligram equivalent protein nitrogen.* We have not been able to ascertain the source of this slight amount of ammonia; but at any rate we are *not* inclined to suppose that it had crystallised out together with the egg-albumin and combined so intimately with the latter that it could not be removed even by washing with the acid sodium chloride solution. We are rather inclined to suppose that we have before us a formation of ammonia by the coagulation of the egg-albumin during the execution of the analysis. In a previous paper[4]) we have touched on this question, and we shall revert to the same later on; here we shall only mention that in all our experiments of this kind we have found such small amounts of ammonia of the same magnitude as above, whatever might be the hydrogen-ion concentration at which the egg-albumin used for the washing had been crystallised. Thus, for instance,

in *Experiment No. 7*, where the egg-albumin was crystallised at a hydrogen-ion concentration of $16.6 \cdot 10^{\div 6}$, we found, after washing, dissolution and coagulation as above, for each milligram equivalent protein nitrogen 0.0027 mg. ammonia nitrogen, — while

in *Experiment No. 9*, where the egg-albumin was crystallised at a hydrogen-ion concentration of $8.1 \cdot 10^{\div 6}$, we found by the same procedure an amount of ammonia of 0.0024 mg. per milligram equivalent protein nitrogen.

Finally, we may mention that in *Experiment No. 9* a part of the precipitate was treated in another way: instead of denaturating the egg-albumin by dissolution and subsequent heating, we treated it with an abundant amount of strong alcohol. After being allowed to stand

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[1]) Comptes-rendus du Laboratoire de Carlsberg **12**, 32 (1916).

[2]) See p. 194, foot-note.

[3]) Comptes-rendus du Laboratoire de Carlsberg **12**, 54 (1916).

[4]) *ibid.* **12**, 60 (1916).

till the next day, the denaturated egg-albumin was filtered off and washed with water. After addition of a slight dose of hydrochloric acid, the alcohol was distilled from the filtrate obtained; a part of the remainder was used for the test for sulphuric acid, which could not be detected, and another part was used for determination of ammonia, by which 0.0018 mg. ammonia nitrogen per milligram equivalent protein nitrogen was found.

It appears from these, as well as from several similar experiments, the details of which may be omitted, firstly that the crystallised egg-albumin washed with a sodium chloride solution prepared as stated by Hopkins does not contain an appreciable amount of sulphuric acid, even if it has been crystallised under such conditions that the crystals before the washing have contained an abundant amount of sulphuric acid. Further it is seen that the slight amount of ammonia which we always found in our analyses of the washed crystallised egg-albumin, is always of the same magnitude, *viz.* abt. 0.002 mg. ammonia nitrogen per milligram equivalent protein nitrogen, whether the egg-albumin had crystallised out with surplus ammonia or not; therefore the ammonia found by the analysis probably does not originate in ammonia or ammonium sulphate having crystallised out together with and firmly combined with the egg-albumin, but it has probably been formed by the denaturation of the egg-albumin.

Accordingly it must be considered a proven fact that *sulphuric acid or ammonia crystallised together with the egg-albumin can be washed away by means of a saturated sodium chloride solution containing 1 % acetic acid, and consequently we are not justified in drawing, from such washing experiments, conclusions as to whether the crystallised egg-albumin contains ammonium sulphate or not.*

Having shown, as above mentioned, that the egg-albumin crystals by washing after Hopkins' method were able to give off sulphuric acid or ammonia, it was an obvious question to ask whether the content of water of the crystals was altered by the washing or not. In order to answer this question we have made the experiment described below.

A greater part[1]) of the precipitate used for Experiment No. 7

[1]) The analyses made later on showed that the amount of protein nitrogen in this part was about 2 g.

crystallised at a hydrogen-ion concentration of $16.6 \cdot 10^{\div 6}$ and then washed after Hopkins' method was mixed up with 700 cc. of the acid saturated solution of sodium chloride, to which 2 gram equivalents of ammonium nitrate (160 g.) per liter had been added[1]). After being shaken repeatedly, the mixture was allowed to stand for two days in a closed flask; it was then filtered, and the ammonia nitrogen and protein nitrogen were determined in weighed samples of the filtrate and the precipitate with adherent mother liquor in just the same way as described in detail under Section A (see p. 170). This experiment is designated as "Experiment I".

"Experiment II" was made at the same time and by an analogous procedure with a similar amount of the washed precipitate used for Experiment No. 7, mixed up with 700 cc. of Hopkins' washing liquid, to which had been added 4 gram equivalents (320 g.) of ammonium nitrate per liter.

The results of the analyses are shown together in Table 38.

TABLE 38.

Number of experiment	100 g. filtrate contained		100 g. precipitate with adherent mother liquor contained			$r = \frac{100\,(a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f}$	Average of r
	Ammonia-N in g. (a_f)	Protein-N in g. (p_f)	Mark of the sample	Ammonia-N in g. (a_b)	Protein-N in g. (p_b)		
I	1.7471	0.0004	I	1.4831	2.1941	6.88_3	6.89_9
			II	1.4797	2.2060	6.93_9	
			III	1.4840	2.1926	6.86_9	
II	3.2846	0.0001	I	2.7985	2.1432	6.90_6	6.90_1
			II	2.7913	2.1771	6.89_9	
			III	2.7910	2.1789	6.89_7	

From Table 38 it appears that the value of r in the case of the washed precipitate is only 6.9, while this factor in the unwashed crystallised egg-albumin has the value 7.86 (see p. 176).

[1]) Even a very abundant addition of ammonium nitrate to Hopkins' washing liquid does not give rise to any separation of salt, and the crystals of egg-albumin are, as later analyses will show, practically insoluble in the washing liquid, even after the addition of the ammonium nitrate.

Now as r is the factor by which the weight of protein nitrogen has to be multiplied to give the weight of egg-hydrate, the low value found for r shows that the precipitate contains less water after the washing than before. Thus, also with regard to r, the composition of the precipitate has undergone an alteration during washing; but from the experiment described it does not appear whether it is the whole of the precipitate that has given off part of its water during washing, or whether it is only a portion of the precipitate that has given off the whole, or the greater part, of its water, while the rest has remained unaltered. We are inclined to suppose the latter alternative to be right, since the precipitate after washing is not clearly soluble in water, but leaves part of the egg-albumin in denaturated insoluble form, and the denaturation is, according to some preliminary experiments to be described in another paper, attended with a loss of hydrate water.

Hopkins has also observed the formation of denaturated egg-albumin when the washed crystals are dissolved in water; but he emphasizes[1]) the fact that the crystals after washing were completely soluble in water, and that the insoluble precipitate does not form at once, but little by little. The last part of Hopkins' statement agrees perfectly with our own observations. We have also found the amount of the precipitate to be considerably increased after some time[2]); but we never had a washed precipitate that was dissolved completely in water. We are therefore inclined to suppose that the denaturation has partly taken place during the washing, while the agglutination of the denaturated precipitate after the dissolving process will only take place by slow degrees, as the hydrogen-ion concentration of the solution obtained is much higher than that corresponding with the optimum point of agglutination of the denaturated egg-albumin. We have through other experiments proved that this hydrogen-ion concentration is also apt to favour a continued denaturation; these experiments will be described in a later paper[3]).

[1]) *loc. cit.*, p. 320.

[2]) In such a mixture of solution and precipitate which had been left to stand for a week, we thus found by a quantitative determination of the amount both of denaturated and of undenaturated egg-albumin, that no less than 48 % of the whole amount of protein nitrogen was present as denaturated egg-albumin.

[3]) Compare also Harriette Chick and C. J. Martin: Journ. of Physiol. **40**, 404 (1910) and **43**, 1 (1911).

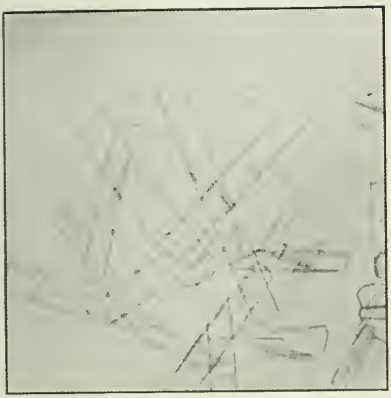

Fig. 18a.

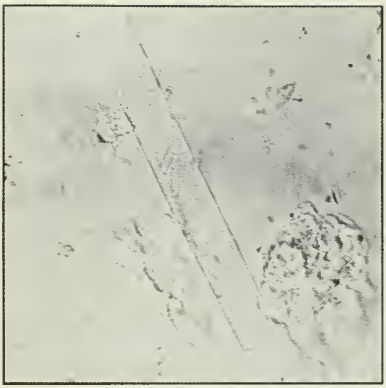

Fig. 18b.

Fig. 19a.

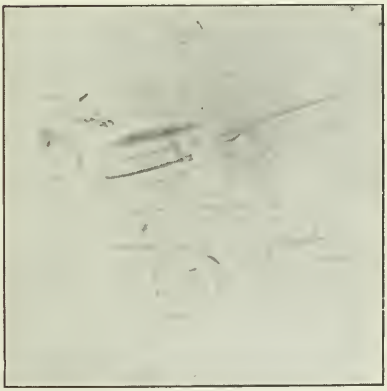

Fig. 19b.

Although we have tried in all details to follow the method of washing employed by Hopkins, it is of course possible that there may exist in a few points some differences between Hopkins' method and ours; it is also possible that serum albumin, which Hopkins, as far as we are able to see from his statements, used for some of his experiments, behaves differently from egg-albumin. In any case we have found — as appears from the above described experiments — that the composition of the crystals is altered during the washing, and further — as seen from Fig. 18 and 19, which represent some microphotos, prepared by Dr. Ö. Winge, of crystals before and after the washing — that also the appearance of the crystals has not remained unaltered during the washing. This alteration, however, is not perceptible with the naked eye, and we can confirm Hopkins' observation (*loc. cit.*, p. 320): "the fine silky lustre of the crystalline precipitate which is seen when it is suspended in the medium and stirred, is still observable when the last trace of sulphate has been washed away."

We then think, from the experiments described in the preceding pages, to be warranted only in drawing the conclusion above mentioned (p. 196), *viz.* that *it is not possible by washing experiments after* Hopkins' *method to decide whether the crystallised egg-albumin contains ammonium sulphate as integral component, or not.*

After the partly negative experimental results recorded above the question arises if it might not be possible in another way to clear up the matter under discussion. We admit that we have not succeeded in furnishing conclusive evidence in any direction; yet we think that the result of some investigations of another nature, which we shall now mention briefly, will be most easily understood if we suppose the crystallised precipitate to contain a slight dose of sulphuric acid.

As a hint in this direction we may mention that it has not been possible to effect the crystallisation of the egg-albumin by means of any other substances than sulphates[1]). Hopkins[2]) writes that he has spent much time in finding such a substance, but he has not succeeded, and many other investigators have pro-

[1]) See Postscript., p. 212.

[2]) *loc. cit.*, p. 319.

bably made similar unsuccessful attempts. We have in our turn made a large number of experiments with several different salts; but it is solely by the use of sulphates that we have obtained the egg-albumin in the crystalline form, and invariably with the same appearance as described in the following section (see p. 209) and easily to be recognised under the microscope. Just as E. G. Willcock[1]), we have thus found magnium sulphate to be applicable for the crystallisation of egg-albumin. On the other hand, the solubility of potassium sulphate in water is so slight that even a saturated solution of potassium sulphate is able to dissolve considerable amounts of egg-albumin; the same applies to sodium sulphate, though in a far less degree. If, however, a saturated solution of a mixture of these two sulphates is used, the egg-albumin will crystallise as easily as by the use of solutions of ammonium sulphate. We have therefore been able to free the egg-albumin solutions from ammonium sulphate by repeated crystallisation and washing of the crystals with a saturated solution of the just-mentioned two sulphates. By such an experiment, for which we used a solution of egg-albumin recrystallised six times by means of ammonium sulphate, and in which we followed the progressive purification from ammonium sulphate by analyses of filtrates, washing liquids and "residues"[2]), we found that the main part of the ammonium sulphate (more than 92 % of the whole amount) was removed in the first crystallisation and washing with the solution of potassium-sodium sulphate. In the second crystallisation and washing, 6.6 % was further removed, and the rest remained in the mother liquor from the third crystallisation. The precipitate was recrystallised three times more, and though it was possible in the mother liquor from these crystallisations to detect ammonia, the quantity was so minimal that it did not even amount to 1 mg. ammonia nitrogen, or $^1/_{20000}$ of the original quantity.

We may further add that by the analysis of the egg-albumin recrystallised six times by means of the solution of potassium-sodium sulphate we found an amount of ammonia corresponding with 0.0019 mg. ammonia nitrogen per milligram equivalent protein nitrogen. It has already been mentioned above (see p. 196) that we are inclined to consider this slight amount of ammonia as formed by the coagulation of the egg-albumin during the execution of the analysis.

From this experiment it appears that the purification from

[1]) Journ. of Physiol. **37**, 32 (1908).

[2]) Comptes-rendus du Laboratoire de Carlsberg **12**, 15 (1916).

ammonium sulphate takes place as easily as the purification of the egg-albumin from the components of the ash, as is mentioned in a previous paper[1]), and consequently we have here doubtless only a replacing of one salt solution by another. The crystals separated have, however, in either case exactly the same appearance both macroscopically and under the microscope, and accordingly their composition is most probably essentially identical, or at any rate analogous. We have not made any determination of the magnitude of the factor r (see p. 176) in the case of the crystals thus obtained; *but as we have not been able to prepare these crystals without use of sulphates, we consider it a plausible supposition that the crystals do not consist solely of pure egg-albumin-hydrate, but also contain the sulphate group* SO_4.

This opinion is confirmed by some investigations, the details of which will be described in the following paper, but the results of which we shall briefly mention as being of importance for the question here dealt with.

Among the factors determining the state of equilibrium between the precipitate crystallised out and the surrounding mother liquor, the hydrogen-ion concentration is — as already mentioned in a previous paper[2]) — one of essential significance. If now, with regard to the crystallisation of egg-albumin, it were a question as to crystallisation of the ampholyte, the egg-hydrate itself, without surplus sulphuric acid or ammonia, we should expect — as is especially emphasized by L. Michaelis[3]) — that the optimum of crystallisation of the egg-albumin would be situated at a hydrogen-ion concentration corresponding with the isoelectric reaction of egg-albumin. This is, however, not the case, since the isoelectric point of egg-albumin is situated at a hydrogen-ion concentration of $15.7 \cdot 10^{\div 6}$, corresponding with $p_{H'} = 4.80$, while the optimum of crystallisation, according to investigations related in the following paper, is situated at a hydrogen-ion concentration of $26—27 \cdot 10^{\div 6}$, corresponding with $p_{H'} = 4.58$.

The egg-albumin crystallised at this hydrogen-ion concentration contains, however, measurable amounts of surplus sulphuric

[1]) *loc. cit.* p. 15.

[2]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 20 (1916).

[3]) Biochem. Zeitschr. **30**, 143 (1910), and **47**, 250 (1912); L. Michaelis, Die Wasserstoffionenkonzentration p. 44 (1914).

acid, which, as is seen in Fig. 17 (see p. 188), may come to about 8 cc. $^{n}/_{1000}$ sulphuric acid per milligram equivalent of protein nitrogen, that is, one equivalent of sulphuric acid per about 125 equivalents of protein nitrogen. Therefore there can hardly be any doubt that, in this case, where the conditions of crystallisation are the best, an egg-albumin sulphate crystallises out, and hence it is highly probable that the crystallised precipitate always contains sulphuric acid. This is not at all at variance with what we have in a previous section found concerning the amount of surplus sulphuric acid or ammonia contained in the crystals (see p. 188).

In fact, if we consider the content of sulphuric acid of the precipitate crystallised at the optimum of crystallisation ($26—27 \cdot 10^{\div 6}$) as the normal amount, it will be seen from Fig. 17 (p. 188) that precipitates crystallised at higher concentrations of hydrogen-ion than the optimal one have carried with them some sulphuric acid, whereas precipitates crystallised at lower hydrogen-ion concentrations have carried along some ammonia. Further, it will appear from the investigations mentioned in the following paper that together with this alteration in the composition of the crystal mass an alteration of the solubility also takes place, since under otherwise like conditions of crystallisation the amount of egg-albumin left in the solution will increase considerably whenever the hydrogen-ion concentration is higher or lower than the optimum. The case is thus quite similar, for instance, to what occurs in the precipitation of a solution of aluminium sulphate by means of a solution of sodium hydroxide. The main product in this process is aluminium hydroxide; but the completeness of the precipitation, and the composition of the precipitate formed, are very largely dependent on the relative proportion of the reacting substances. If a deficient amount of sodium hydroxide is added, the precipitation is incomplete, and the precipitate separated contains sulphuric acid, while an excess of sodium hydroxide will produce the effect that a portion of the precipitate is dissolved as sodium aluminate and that the undissolved remainder contains alkali.

The facts above set forth seem to us to make it very probable that the precipitate crystallised out is a sulphate which in crystallising has withdrawn an amount of sulphuric acid or ammonia dependent on the hydrogen-ion concentration; about this amount of sulphuric acid or ammonia it is possible to form a

fairly correct estimate by means of Fig. 17. These investigations, however, give no information as to whether the crystals contain also ammonium sulphate, or not. P. Pfeiffer and his collaborators[1]) have in a very interesting series of works investigated the behaviour of some of the common amino-acids and polypeptides towards salts, and they have shown the existence of several well-defined compounds of these ampholytes and the salts used. It seems natural on this basis to draw analogous conclusions concerning the composition of the products obtained by precipitation or crystallisation of proteins by addition of salt. It is, of course, not possible in this manner to furnish a direct proof, and we have neither in Pfeiffer's work nor in the rest of the literature nor in our own experimental results been able to find any decisive evidence that the egg-albumin crystals contain ammonium sulphate as an integral component. We must, as already mention in Section A (p. 178), consider it probable that egg-albumin crystals are either ammonium sulphate free or only contain quite minimal amounts of the latter substance.

On the basis of the investigations recorded in this paper we thus think to be able to answer the question concerning the composition of the egg-albumin crystals as follows:—

The egg-albumin crystals consist essentially of egg-albumin and water, and the water-content of the crystals is within wide limits independent of the conditions under which the crystallisation takes place: for 1 g. egg-albumin about 0.22 g. water is present.

Besides egg-albumin and water, the crystals probably always contain a little sulphuric acid, and must therefore be considered as consisting of a hydrous egg-albumin sulphate. The normal sulphuric acid content of the crystals cannot be stated exactly: but it probably amounts to 1 equivalent of sulphuric acid per about 125 equivalents of protein nitrogen.

A hydrous egg-albumin sulphate of this composition is most likely only separated at the hydrogen-ion concentration optimal for the crystallisation ($h = 26—27 \cdot 10^{-6}$; $p_H = 4.58$). At higher hydrogen-ion concentrations, the crystals contain more sulphuric acid;

[1]) P. Pfeiffer and J. v. Modelski: Zeitschr. physiol. Chem. **81**, 329 (1911), **85**, 1 (1913); P. Pfeiffer and Fr. Wittka: Ber. d. deutsch. chem. Ges. **48**, 1041, 1289 and 1938 (1915); P. Pfeiffer and J. Würgler: Zeitschr. physiol. Chem. **97**, 128 (1916).

what has taken place is possibly a formation of another salt which is richer in sulphuric acid, since the crystals assume a more pointed form (see p. 210); besides, the crystallisation is less complete, the solubility of the crystals being thus greater than it is at the optimum of crystallisation. *At hydrogen-ion concentrations lower than that corresponding with the optimum of crystallisation, the content of surplus sulphuric acid of the crystals is smaller than the above stated amount* (one equivalent of sulphuric acid per about 125 equivalents of protein nitrogen). The most probable explanation seems to be that *under these circumstances a little ammonia has been retained by the crystals.* The shape of the latter is the normal one (see p. 209); but the solubility is greater than it is at the crystallisation optimum.

The egg-albumin crystals probably do not contain ammonium sulphate as an integral component: but the ammonia retained by the egg-albumin sulphate may, of course, within certain limits be considered as being present in the form of ammonium sulphate.

In this case it will be easy to calculate the quantity y, which is the factor by which the weight of the protein nitrogen of the crystals has to be multiplied to give the weight of the ammonium sulphate present in the crystals. If the crystallisation, for instance, takes place at a hydrogen-ion concentration of $13 \cdot 10^{\div 6}$, the crystals do not contain either surplus sulphuric acid or ammonia (see Fig. 17), and this means — according to what has been stated above — that an amount of ammonia equivalent to the normal content of sulphuric acid of the crystals has been retained, — 1 equivalent of ammonia nitrogen for about 125 equivalents of protein nitrogen. Now as the weight of ammonia nitrogen has to be multiplied by 4.7163 to give the corresponding weight of ammonium sulphate, we have

$$125 \cdot y = 4.7163$$

and

$$y = \text{abt. } 0.04$$

It thus appears — if our assumptions are correct — that y is quite a minimal quantity, and the case is similar as to the product $s \cdot y$ (see p. 170). The error committed in disregarding these quantities is therefore of no essential significance; but — as above emphasized — it must not be forgotten that on this point it has not been possible to attain conclusive results.

D. Remarks concerning the Character of the Crystallising Process and the Form of the Crystals.

Although the precipitation of proteins by addition of salt has been the object of numerous investigations, we are still far from having arrived at a clear insight into the character of the process of precipitation. The interpretation most generally adopted is probably that advocated especially by K. Spiro[1]), according to which the salt precipitation of the proteins is really a distribution between two phases: one rich in water and salt, but poor in protein, and the other poor in water and salt but rich in protein. This latter phase, which represents the precipitate, often consists of fine drops, which more or less rapidly — according to the existing conditions — grow stiff, forming masses resembling globules. Now as there is surely no essential difference between the crystallising process of egg-albumin by means of ammonium sulphate and the precipitation of an amorphous precipitate at a somewhat higher concentration of ammonium sulphate, it will be natural to consider also the crystallising process as a phase division. This interpretation is supported by the fact that the egg-albumin of impure solutions often crystallises in small needles arranged as globules; but this fact can by no means be taken as a proof. In support of this interpretation the fact may further be alleged that the egg-albumin, even if purified by repeated recrystallisations, may be separated in the form of oil, if the hydrogen-ion concentration is higher than that optimal to the crystallisation, and that this oil may little by little assume a crystalline form. To this, however, the objection may be made that in this case — as already mentioned above — we have a crystallisation of a mixture of the ordinary egg-albumin sulphate and a compound richer in sulphuric acid.

According to our experience, no phase-division of the above mentioned nature takes place in a normal crystallisation of egg-albumin, and we have not been able either macroscopically or microscopically to observe a formation of drops before crystallisation. If to an egg-albumin solution we add so much of a strong solution of ammonium sulphate that, after stirring, a little amorphous precipitate remains, and if this precipitate be filtered off, we obtain a clear filtrate which has completely the character

[1]) Beiträge zur chem. Physiol. und Patholog. **4**. 300 (1904).

of a supersaturated solution. By inoculating and stirring, it will rapidly crystallise; but without inoculation it may remain clear for hours, and may then without visible cause commence crystallising. Altogether there seems to be *no reason for considering the crystallisation of egg-albumin to be essentially different from any other crystallisation process; in our view, we have here merely the crystallisation of a supersaturated solution of a substance crystallising with difficulty and slowly.*

F. G. Hopkins[1]), through his researches on the preparation of pure egg-albumin by crystallisation with ammonium sulphate, arrived at a similar opinion: "I have frequently observed, in cases in which the process has appeared slower than usual, that slight shaking of the flask has been quickly followed by complete separation. On one occasion a solution of the product at this stage, to which ammonium sulphate had been added in quantity too small to produce any definite precipitate, remained, while undisturbed, practically clear for no less than three weeks. On then shaking the flask a bulky precipitate wholly composed of crystals separated within an hour. The mixture behaved as a supersaturated solution." (*loc. cit.*, p. 311).

Thus we cannot indorse the theory of Fr. N. Schulz[2]), according to which there is an essential difference between the crystallisation of proteins and other crystallisations. Schulz' argumentation appears most clearly from his own statements (*loc. cit.*, p. 40): "Die Veranlassung zur Krystallisation ist in den beschriebenen Fällen die Entziehung der Lösungsbedingungen. Diese Entziehung ist keine allmähliche; es geht der krystallinischen Abscheidung immer eine wesentliche Übersättigung voraus. Die Abscheidung erfolgt ziemlich plötzlich und in viel reichlicheren Mengen, als sie der Entziehung der Lösungsbedingung, die zur Abscheidung geführt hat, entspricht.

Bei der Darstellung des krystallinischen Eieralbumins nach der Ammonsulfatmethode können z. B. wenige Tropfen concentrirter Ammonsulfatlösung genügen, um die schliessliche Ausfällung des krystallisirenden Bestandtheils aus mehreren 100 ccm einer Lösung zu bewirken, die ohne Zusatz dieser wenigen Tropfen dauernd klar geblieben wäre. Durch diese minimale Concentra-

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[1]) Journ. of Physiol. **25**, 306 (1900).

[2]) Die Krystallisation von Eiweissstoffen, Jena 1901.

tionserhöhung werden nicht nur Spuren von Eiweiss zur Krystallisation gebracht, sondern grosse Mengen. Namentlich beim Umkrystallisiren zeigt es sich, dass durch den geringen Mehrgehalt an Salz der krystallisirende Bestandtheil fast quantitativ aus seiner Lösung entfernt wird. Auf diesen Umstand ist es auch zurückzuführen, dass es nicht gelingt durch allmähliches Erhöhen der Concentration grosse Krystalle zu züchten, denn trotz aller Vorsicht erfolgt die Krystallisation plötzlich.

Es besteht hier ein eklatanter Unterschied gegenüber den meisten anderen Krystallisationen, bei denen, falls die Krystallisation einmal begonnen hat, die Krystallabscheidung bis zu einem gewissen Grade proportional der Entziehung der Lösungsbedingungen ist."

Thus Schulz clearly realises that the crystallisation is preceded by a stage in which the solution must be considered as supersaturated; but as for the rest, the facts set forth by him are not quite correct, or they are at any rate not correctly explained. Thus, for instance, it is correct that a few drops of a saturated solution of ammonium sulphate may give rise to an incipient crystallisation of several hundreds of cubic centimeters of an egg-albumin solution provided that the solution previously contains an abundant amount of ammonium sulphate; but such a solution will not, as Schulz writes that it does, without these few drops remain clear. It will, as is mentioned more closely in the following paper, crystallise in the course of a shorter or longer time; but the velocity of crystallisation depends on the degree of supersaturation. Therefore Schulz is not quite right in asserting that a slight increase of the content of salt may bring about a fairly quantitative separation of the crystallising substance. On the contrary, the increase of the content of ammonium sulphate has proved to be followed by a *continually* increasing separation of the crystals. In order to prove this, we must, however, take into consideration not solely how much albumin the mother liquor contains after the crystallisation, but also at what time the crystallisation really has come to an end; in other words, we must tako into account the velocity of crystallisation, which increases very considerably with increasing concentration of salt. *Therefore the difference asserted by* Schulz *between the crystallisation of proteins and a common crystallisation does not exist in the case of egg-albumin.* Indeed — in contradistinction to Schulz' state-

ment — we as well as other experimenters have succeeded, without difficulties of any kind, in preparing fairly large-sized crystals of egg-albumin by slow crystallisation, the ammonium sulphate solution being added little by little in the course of some days or weeks.

We are therefore of opinion that the crystallisation of egg-albumin must be looked upon as a common crystallisation, and we shall in this connection make only a few supplementary remarks on the water contained in the egg-albumin crystals. We have not made any experiments with a view to ascertaining whether the water existing in the crystals is to be considered as water of constitution or of crystallisation or as imbibed water[1]), the question being irrelevant in this connection. Yet we would draw attention to the fact that Harriette Chick and C. J. Martin[2]) have found that egg-albumin precipitated by an abundant amount of ammonium sulphate may, if submitted to a high pressure, give off a part of the water contained in it. The precipitate separated was first pressed between filter paper, then placed between filter paper surrounded by kieselguhr, and finally submitted to a pressure of about three tons to the square inch. It then appeared that the liquid squeezed out contained comparatively more water and less ammonium sulphate than the mother liquor, from which result Chick and Martin concluded that the "imbibed water" present in the egg-albumin precipitated had been squeezed out of the precipitate.

We have made a few experiments by a similar procedure on crystallised egg-albumin, but we have not been able to detect any squeezing out of water. We have analysed both the mother liquor and the liquid expressed, which we collected in 9 fractions. The ratio of water to ammonium sulphate was found to be fairly alike in the mother liquor and in all the nine fractions, and we could not detect any indication of water having been squeezed out of the crystals.

In conclusion, we shall briefly mention a few experiments which we have made in order to prepare well-formed egg-albu-

[1]) As to the difference between water of crystallisation and imbibed water, the reader is referred to an interesting paper by J. R. Katz: "Untersuchungen über die Bindung des Quellungswassers in quellbaren Krystallen" (Zeitschr. physiol. Chem. **95**, 1 (1915).

[2]) Biochem. Journ. **7**, 391 (1913).

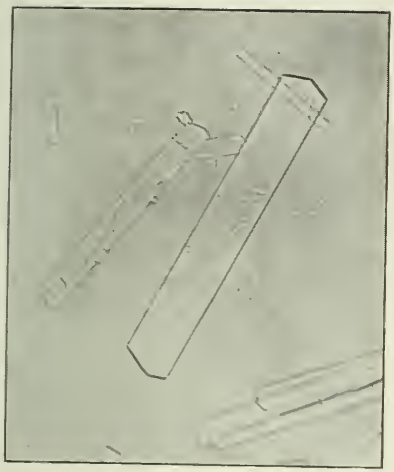

Fig. 20

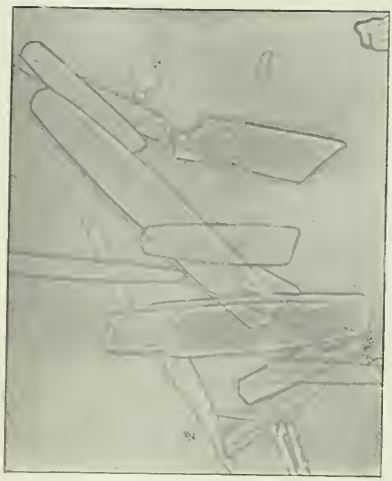

Fig. 21

Fig. 22

Fig. 23

min crystals under different conditions of crystallisation. The results obtained agree mainly, but not in all details, with E. Artinis' statements in St. Bondzynski and L. Zoja's paper[1]) and with A. Wichmann's later investigations[2]).

We have made four main experiments of this kind and designate them by the numbers 1 to 4; Professor O. B. Bøggild has been kind enough to submit the four samples to a crystallographic investigation, and we are indebted to Dr. Ø. Winge for some microphotos of the crystal samples.

Experiment No. 1. To 350 cc. of an egg-albumin solution containing ammonium sulphate from which the main part of the egg-albumin has already crystallised out and been filtered off, a saturated ammonium sulphate solution, 5 cc. in all, was added in small portions, during the course of three weeks, the mixture being well stirred on each addition. The mixture was now left to stand nine weeks more for crystallisation. An analysis showed that the solution per 100 g. water contained

1.104 g. egg-hydrate[3])
and 26.620 g. ammonium sulphate.

Thus we had here a *crystallisation of a solution poor in protein but rich in ammonium-sulphate.*

Experiment Nr. 2. In this case the *crystallisation took place in the course of four days in a solution rich in protein but poor in ammonium sulphate*, since the analysis showed that per 100 g. water

22.879 g. egg-hydrate
and 21.404 g. ammonium sulphate were present.

While these two experiments were made on solutions to which neither surplus sulphuric acid nor ammonia were added (Experiment No. 1: p_H = about 5.0; Experiment No. 2: p_H = about 4.8),

a little surplus ammonia was present in the solution used for *Experiment Nr. 3* (p_H = about 5.3), and in that used for *Experiment Nr. 4 a little surplus sulphuric acid* (p_H = about 4.4) was present.

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[1]) Zeitschr. physiol. Chem. **19**. 5 (1893).
[2]) *Ibid.* **27**, 575 (1899).
[3]) The value 7.86 is used for the factor r (see p. 176).

In Experiment Nr. 3

8.594 g. egg-hydrate
and 25.959 g. ammonium sulphate

per 100 g. water were present, while we have made no exact analysis of the solution used for Experiment Nr. 4; but the content of egg-albumin was in this case about 4.3 g. per 100 g. water; the time of crystallisation was in either case about three weeks.

It appears clearly from Fig. 20 to 23 — Fig. 20 corresponds with Experiment No. 1, Fig. 21 with No. 2, Fig. 22 with No. 3 and Fig. 23 with No. 4 —, which represent the microphotos of the crystals obtained, that the crystals separated at the highest hydrogen-ion concentration (p_H = about 4.4 (Experiment No. 4)) have a more pointed shape than the rest of the crystals.

The declaration of Professor Bøggild as to the crystallographic examination is worded as follows:

"The crystals are in all the four preparations uniformly developed; they are apparently rhombic and have the form of very regular rulers; the dimensions of the greater crystals are about $0.2 \cdot 0.015 \cdot 0.001$ mm. At the ends they are bounded by a dome, the planes of which form an angle of about 40^0 with each other (with the longitudinal direction of the crystals, an angle of about 70^0). The crystals are colourless; the refraction is considerably greater than that of the surrounding liquid; not even the slightest trace of double refraction is perceptible."

Thus there can hardly be any doubt that the crystallisation of egg-albumin really means a separation of well-developed, measurable crystals characteristic of the said substance. This fact supports our interpretation of the egg-albumin crystallisation as a process of quite the same nature as a common crystallisation.

SUMMARY.

Section A.

(1) By means of a method based upon the principle of the "Method of Proportionality", it is proved that the crystals of egg-albumin contain considerable amounts of water, *viz.* about 0.22 g. water per one g. egg-albumin, the factor by which the weight of

protein nitrogen has to be multiplied to give the weight of the crystals being about 7.86, while the factor by which the weight of protein nitrogen has to be multiplied to give the weight of the water-free egg-albumin is only about 6.4.

(2) The amount of water in the egg-albumin crystals is — within rather wide limits — independent of the conditions of crystallisation (time and temperature of crystallisation, concentrations of ammonium sulphate, protein and hydrogen-ions).

Section B.

(1) It is explained how it is possible, by means of the results obtained in the preceding paper as to the capacity of the egg-albumin to combine with acids and bases, and by means of the principle of the "Method of Proportionality", to determine the amount of surplus sulphuric acid or ammonia which may be contained in the egg-albumin crystals.

(2) If the crystallisation of egg-albumin takes place at a hydrogen-ion concentration of about $13 \cdot 10^{\div 6}$, the crystals separated do not contain either surplus sulphuric acid or ammonia; at higher hydrogen-ion concentrations than about $13 \cdot 10^{\div 6}$ the crystals are separated with surplus sulphuric acid, at lower concentrations with surplus ammonia, and the contents of these two substances are the greater, the higher or lower, respectively, the hydrogen-ion concentration is.

(3) At a hydrogen-ion concentration of $13 \cdot 10^{\div 6}$, the egg-hydrate contains before crystallisation neither surplus sulphuric acid nor ammonia, and it crystallises — as mentioned *sub* (2) — likewise without surplus sulphuric acid or ammonia. At a higher hydrogen-ion concentration than about $13 \cdot 10^{\div 6}$ the egg-albumin crystallises with a *somewhat smaller* amount of surplus sulphuric acid and at lower hydrogen-ion concentrations with a *somewhat smaller* amount of surplus ammonia, than it contains before crystallisation.

Section C.

(1) By means of several experiments it is proved that sulphuric acid or ammonia which has crystallised out together with the egg-albumin may be removed by repeated careful washing of the crystals with Hopkins' solution (a saturated sodium chloride solution containing 1 % acetic acid).

(2) On the basis of the experimental results mentioned under (1) together with other facts observed in connection with the washing of the crystals, we think to be justified in maintaining that it is not possible by means of washing experiments by Hopkins' method to decide whether the crystallised egg-albumin contains ammonium sulphate as an integral component, or not.

(3) The fact that it has not been possible to prepare crystals of egg-albumin without using sulphates, goes to show that the crystals do not consist solely of pure hydrous egg-albumin, but that they further contain the sulphate group, SO_4, in slight quantity. This interpretation is supported by investigations of another nature, which are thoroughly described.

(4) In the concluding part of the section it is discussed, whether and how it is possible, on the basis of the investigations reported in the present paper, to answer the question as to the composition of the egg-albumin crystals.

Section D.

contains some considerations on the character of the crystallisation process and the shape and other properties of the crystals separated, and from these considerations we draw the conclusion that there does not appear to be any reason for considering the crystallisation of egg-albumin to be of a different nature from crystallisation processes in general; in our opinion it is simply the crystallisation of a supersaturated solution of a substance crystallising slowly and with difficulty.

March 1917.

POSTSCRIPT.

(Added in the proof.)

After the manuscript of the above paper was finished, we have succeeded in separating egg-albumin in crystalline form by means of a proper mixture of primary and secondary ammonium phosphates. This crystalline precipitate, which is probably a phosphate, exhibits under the microscope a similar appearance to that above described precipitated by means of ammonium sulphate; but a closer examination of it is far from being completed.

September 1917.

STUDIES ON PROTEINS.

IV. ON THE STATE OF EQUILIBRIUM BETWEEN CRYSTALLISED EGG-ALBUMIN AND SURROUNDING MOTHER LIQUOR, AND ON THE APPLICABILITY OF GIBBS' PHASE RULE TO SUCH SYSTEMS.

BY

S. P. L. SØRENSEN AND MARGRETHE HØYRUP.

THE first systematic investigation into the state of equilibrium between crystallised egg-albumin and the surrounding ammonium-sulphate-containing mother liquor was made by G. Galeotti and his collaborators, who published a series of works on the state of equilibrium between proteins and electrolytes[1]).

In the third of the papers cited, Galeotti deals with the system: egg-albumin, ammonium sulphate, water. The egg-albumin used for the experiments was purified through precipitation by ammonium sulphate, dissolution and reiterated precipitation; these operations were repeated a few times; but as no crystallisation took place, Galeotti's egg-albumin must have contained abundant quantities of conalbumin. The egg-albumin thus purified was dissolved in water, and the solution was left to stand in a dish over concentrated sulphuric acid, till an abundant crystallisation of the egg-albumin had taken place; then the mixture was filtered and a part of the filtrate analysed. The rest of the filtrate, after addition of water and ammonium sulphate, was left to stand for further crystallisation. When the latter had avanced sufficiently far, the mixture was again filtered and a part of the filtrate analysed, while the rest, after addition of water and ammonium sulphate, was again left to stand for crystallisation etc.

[1]) G. Galeotti: Zeitschr. physiol. Chem. **40**, 492 (1903); **42**, 330 (1904); **44**, 461 (1905); G. Guerrini: *ibid.* **47**, 287 (1906); G. Galeotti: *ibid.* **48**, 473 (1906); S. La Franca: *ibid.* **48**, 481 (1906) and V. Scaffidi: *ibid.* **52**, 42 (1907).

The analyses were made in the following manner: — the measured or weighed samples of the filtrate were evaporated and dried to constant weight at 100°; the dry residue was treated with hot water, by which treatment only the egg-albumin was left undissolved. It was then possible to determine the amount of egg-albumin by filtration on a filter previously dried and weighed, washing and subsequent drying to constant weight; the amount of ammonium sulphate was determined in the filtrate by precipitation with barium chloride, and finally, the amount of water was calculated as the difference. In this way Galeotti was able to determine the composition of the mother liquor, which in each case at the experimental temperature (15°) was equilibrious to the crystallised egg-albumin.

Galeotti now supposes that the system is completely determined at a given temperature and given concentration of ammonium sulphate. This view is in full accordance with Gibbs' Phase Rule, in accordance with which a three-phased system consisting of three components is completely determined at a given concentration of one of the components. The case is quite different in the experiments made by G. Guerrini and described in the fourth of the papers above cited. This investigator finds that a solution of egg-albumin and sodium sulphate equilibrious to both the precipitated egg-albumin and the deca-hydrate of sodium sulphate at a given temperature may have a varying composition. We shall not discuss more closely the considerations set forth by V. Henri[1]) and G. Galeotti[2]), according to which the phase rule of Gibbs is not applicable in the case of colloidal solutions. The considerations are certainly justified when we are dealing with suspensoid systems, whereas egg-albumin solutions — as has been repeatedly pointed out in the previous papers — may be most properly described as real solutions of a very complicated ampholyte. Such an interpretation requires, however, that Gibbs' phase rule should also be applicable to egg-albumin solutions. We think it appears from the experiments described in this paper that this is really the case, and if the results of Galeotti and his collaborators are sometimes at variance with this interpretation, it may be easily accounted for.

[1]) Zeitschr. physik. Chem. **51**, 31 (1904).
[2]) *Ibid.* **54**, 727 (1906).

In fact, Galeotti has taken it for granted that, for instance, in the precipitation of an egg-albumin solution with ammonium sulphate, he was dealing with a system consisting of three components — water, ammonium sulphate and egg-albumin; this is, however, as will be seen from our previous papers, only correct in certain particular cases. Generally the ammonium sulphate used does not contain ammonia and sulphuric acid in equivalent quantities, and the same applies to an egg-albumin solution purified by re-precipitation or re-crystallisation and perhaps subsequent dialysis. But these very traces of sulphuric acid or ammonia, the presence of which can be proved and the quantity of which can be determined through measurements of hydrogen-ion concentrations, are conclusive for the properties and reactions of the egg-albumin solutions. This circumstance is also of essential importance for the state of equilibrium between the egg-albumin crystallised out and the surrounding mother liquor, as will appear clearly from the experiments hereinafter described. There is nothing remarkable in this, but the point must naturally also be borne in mind when considering the question as to whether Gibbs' phase rule is applicable, or not, to the systems under discussion. The fact is, that if the circumstance just mentioned be taken into account, the number of components of the system will not be three, but four, and according to the phase rule a three-phased system consisting of four components has three degrees of freedom. This means, in other words, that a system consisting of egg-albumin crystallised out and equilibrious to the surrounding mother liquor at a given temperature is only completely determined when the concentration of two of the components of the system is given. In accordance herewith we have found, by the investigations mentioned below, that if the crystallisation of the egg-albumin takes place at a given temperature and at given concentrations of ammonium sulphate and hydrogen-ions, the content of egg-elbumin of the mother liquor equilibrious to the crystals will always be the same, within the limits of the experimental errors.

As already mentioned in the preceding paper[1]), Galeotti believes his experiments justify the conclusion that crystallised

[1]) Comptes-rendus du Laboratoire de Carlsberg **12**. 167 (1917).

egg-albumin does not contain either ammonium sulphate or water. This conclusion — which, judging by our experiments described in the preceding paper, is surely incorrect — will not have any influence on the consideration of the questions dealt with by Galeotti and his co-workers. On the other hand, the circumstance is of essential significance for the comprehension of another question in which several investigators have been engaged in the course of time, that is, the question as to the influence of the protein concentration on the state of equilibrium in the process of »salting out«. If the phase rule is applicable to the systems under consideration, the state of equilibrium must of course be independent of the initial concentration of protein. From a concentrated egg-albumin solution, abundant quantities of egg-albumin must be precipitated or crystallised out by addition of, for instance, ammonium sulphate solution; from a diluted egg-albumin solution, on the contrary, only small quantities. But the amount of egg-albumin remaining in the solution must be the same in either case, if the conditions are otherwise equal, and this means especially: if the temperature, ammonium sulphate and hydrogen-ion concentration of the mother liquor are equal in both cases.

According to the common view, however, protein solutions do not behave in the way demanded by the phase rule, since the protein, under otherwise like conditions, is supposed to be salted out the more completely the greater the starting concentration of the solution.

It is not necessary to discuss more closely the whole literature relating to this question: we may confine ourselves to elucidating the question by means of a single example. For this purpose we have chosen Harriette Chick and C. J. Martin's[1]) remarkable work on the precipitation of egg-albumin by means of ammonium sulphate, as they are here dealing with experimental conditions which are surely analogous to those of our experiments. In a series of experiments, the details of which will be stated in the Experimental Part (see p. 248), Chick and Martin investigate into the influence of the starting concentration of egg-albumin on the precipitation of egg-albumin by ammonium sulphate. In all the experiments of the series, the ratio of ammonium sul-

[1]) Biochemical Journal **7**, 380 (1913).

phate to water was constant, *viz.* 31 : 100; but the amount of albumin varied in the experimental mixtures from 3.3 g. to 27.4 g. egg-albumin per 100 g. water. Now Chick and Martin find that the egg-albumin in these experiments is precipitated the more completely as the starting concentration increases. The error committed by Chick and Martin is, that they have considered it a fact that the ratio of ammonium sulphate to water does not undergo any alteration during the precipitation, and consequently they have not undertaken any determination of the amount of ammonium sulphate of the filtrates. There can, however, in our opinion, hardly be any doubt that hydrous egg-albumin is separated by this precipitation just as by crystallisation, so that the ratio of ammonium sulphate to water is different in the filtrate from what it is in the original mixture. Now it is obvious that with an increasing initial concentration of egg-albumin, an increasing amount of water will be removed from the solution by the precipitation, and the ammonium sulphate concentration of the filtrate will consequently also be increased. As an increase of ammonium sulphate concentration causes a more complete precipitation of the egg-albumin, the experimental results of Chick and Martin become explicable. As more closely mentioned in the experimental part (see p. 249), we have recalculated these results, considering it a fact that precipitated and crystallised egg-albumin have the same composition; it appeared by this recalculation that the curious facts found by Chick and Martin are probably — as might be expected from what is stated above — entirely attributable to the different ammonium sulphate concentration of the filtrates, but have nothing to do with the starting concentration of the protein. In accordance herewith we have, by our experiments made in order to elucidate this question, found that the amount of albumin left in the mother liquor after the crystallisation of the egg-albumin is independent of the starting concentration of the protein, if only the ammonium sulphate concentration of the mother liquor at the end of the crystallisation is the same in all the experiments, and that this is really the fact must of course be proved analytically.

Hence it appears that the above mentioned fact can be accounted for without any difficulty, and does not preclude the application of the phase rule to such systems as those inves-

tigated by us. In our opinion, it is highly probable that the same will hold good for several other precipitations of proteins, and the circumstance that the contrary view has hitherto prevailed must be due to a deficient experimental treatment of the question.

In the experiments described in the following sections, our object has been the same as in our preceding papers, namely to try to ascertain whether solutions of egg-albumin behave, and may be advantageously considered, as real solutions, and especially we have — as is seen from the above historical remarks — directed our attention to the question as to the applicability of Gibbs' phase rule to such systems as those inquired into by us. With this object in view we have tried to obtain information as to the state of equilibrium between the egg-albumin crystallised out and the surrounding mother liquor; but it has not been our intention through these investigations to procure such exhaustive experimental material as would render it possible to construct complete diagrams of the state of equilibrium under any conditions of crystallisation. On account of the great number of factors which exert influence on the state of equilibrium, a very extensive experimental material would be necessary for the construction of such diagrams, and furthermore, it is to be noted that it is very difficult to procure a final and complete state of equilibrium in the case of such a slowly crystallising matter as egg-albumin. We have, however, as will appear from what follows, been of opinion that we were able to attain our object without absolutely exhaustive treatment of the subject, for which purpose we have tried to form an idea of the influence exerted by each factor, both on the state of equilibrium itself and on the velocity with which this state sets in. Furthermore, we have tried to arrange our experiments in such a manner that we were able in different ways to make sure that we had here to deal with real states of equilibrium, which by proper alterations in the conditions of crystallisation can be displaced in two opposite directions.

In the following experimental sections we shall have to inquire separately into the significance of each factor for the velocity of crystallisation of egg-albumin on the one hand, and on the other, for the state of equilibrium between the precipitate crystallised and the surrounding mother liquor.

Section A will discuss the significance of the ammonium sulphate concentration;

Section B the influence of the temperature;

Section C the significance of the hydrogen-ion concentration, and

Section D that of the protein concentration in the above mentioned respect.

A. The Influence of the Ammonium Sulphate Concentration on the Velocity of Crystallisation of Egg-albumin and on the State of Equilibrium between the Precipitate crystallised and the surrounding Mother Liquor.

Series of experiments a. In the six experiments belonging to this series, which were made in February 1914, all the experimental mixtures were prepared in such a way that the ratio of egg-albumin to water was constant, and about 10.5 g. egg-albumin-hydrate per 100 g. water, while only the contents of ammonium sulphate were varied (from about 24 to about 30 g. ammonium sulphate per 100 g. water). In order to prepare solutions of such a composition, equal quantities of an ammonium-sulphate-containing solution of egg-albumin, of exactly known composition, were weighed off for each experiment, and to these samples were added different, previously calculated quantities of water and of a strong solution of ammonium sulphate of known composition. After mixing up carefully, crystallisation was induced by adding six drops of inoculating material. The experimental mixtures were then left to stand at ordinary temperature (abt. 18^0) in conical flasks closed by rubber stoppers, and the flasks were well shaken several times every day. After the lapse of four days, one half of each experimental mixture was filtered, and after thirteen days the rest was filtered. The filtrations were effected under the precautions mentioned in a previous paper[1]), in order to avoid evaporation, and the first part of the filtrate was thrown away. In weighed portions of the filtrates the amounts of both protein nitrogen and ammonia nitrogen were determined as earlier described[2]), and the hydrogen-ion concentration of the filtrates was measured electrometrically.

[1]) Comptes-rendus du Laboratoire de Carlsberg **12**. 170 (1917).

[2]) *loc. cit.*, p. 171.

In calculating the composition of both the filtrates and the original experimental mixtures, we have in all the experiments described in this paper reckoned that all protein nitrogen was present in the form of egg-hydrate of the same composition as the precipitate crystallised out, so that the weight of the egg-hydrate present was to be obtained by multiplying the protein nitrogen by 7.86[1]). This method of calculating is especially suited to give a good general view of the question, since the ammonium sulphate concentration (in g. per 100 g. water) of the solution does not undergo any alteration during crystallisation, if the egg-albumin has also in dissolved form combined with the amount of water with which it crystallises later on

To explain more closely the method of calculation, we may here state the calculation of a single analysis (Table 39, Experiment No. 1, analysis of the filtrate after 4 days' crystallisation): The mean values of the nitrogen determinations gave the result that 100 g. filtrate contained

9.44 mg. protein nitrogen
corresponding with $0.00944 \cdot 7.86 =$ 0.074 g. egg-hydrate, and
4.9092 g. ammonia nitrogen
corresponding with $4.9092 \cdot 4.7163 =$ 23.153 g. ammonium sulphate;
so that 100 g. filtrate contained 76.773 g. water.

100.000 g.

Thus the filtrate contained per 100 g. water { 0.096 g. egg-hydrate
30.158 g. ammonium sulphate.

The experimental results are set forth in Table 39 and graphically represented in Fig. 24, where the contents of the filtrates of ammonium sulphate and of egg-hydrate — expressed in g. per 100 g. water — are used as abscissæ and ordinates, respectively; Curve I corresponds with a time of crystallisation of four, and Curve II of thirteen days.

From the second, sixth, seventh and eighth vertical columns of Table 39, as well as from the Curves I and II of Fig. 24, it appears — as might be expected — that *the crystallisation begins and proceeds the more rapidly and completely as the concentration of ammonium sulphate increases.* The two last vertical columns of Table 39 give information about the hydrogen-ion concentration of the filtrates, which on an average corresponds with $p_H =$

[1]) *loc. cit.*, p. 176.

Each experimental mixture contained, before crystallisation, in 300 cc. abt. 25 g. egg-hydrate, or abt. 10.5 g. egg-hydrate per 100 g. water.

No. of exp.	Remarks as to crystallisation.	Time of crystallisation, in days	100 g. filtrate contained		*Thus the filtrate contained per 100 g. water*			Hydrogen-ion concentration of the filtrate (after four days' crystallisation)	
			Protein-nitrogen in mg.	Ammonia nitrogen in g.	*Egg-hydrate* in g.	Ammonium-sulphate in g.	*Mean value of the contents of ammonium sulphate in* g.	$h \cdot 10^6$	p_H
1	On mixing, a voluminous amorphous precipitate appeared at once, and in the course of $^1/_2$ hour the whole formed a pappy mass. The next day the precipitate appeared under the microscope partly as diminutive crystals and partly as amorphous matter.	4	9.44	4.9092	0.096	30.158	30.177	15.28	4.816
		13	8.54	4.9142	0.087	30.196			
2	On mixing, a rather voluminous amorphous precipitate was formed at once; the next day it exhibited under the microscope a quite homogeneous appearance: exceedingly small, but distinct crystals.	4	15.43	4.7998	0.157	29.307	29.337	14.13	4.850
		13	14.32	4.8077	0.146	29.367			
3	On mixing, no permanent amorphous precipitate was formed; but crystallisation soon began, and in the course of $^3/_4$ hour an abundant crystalline precipitate had been formed, exhibiting under the microscope the normal appearance.	4	66.14	4.4622	0.663	26.831	26.829	13.96	4.855
		13	62.93	4.4629	0.631	26.827			
4	Crystallisation began in the course of $^1/_2$ hour and rapidly increased; the precipitate appeared under the microscope as consisting of nice crystals.	4	114.2	4.3084	1.140	25.793	25.784	13.52	4.869
		13	110.6	4.3073	1.103	25.775			
5	It was not until the lapse of one hour, and after repeated shaking, that the crystallisation began, and only after five hours had an abundant precipitate formed, the precipitate exhibiting und. the microscope large well-formed needles.	4	177.0	4.1630	1.761	24.861	24.886	13.46	4.871
		13	170.3	4.1720	1.695	24.911			
6	The crystallisation began after one hour and repeated shaking; but even after five hours it was anything but abundant. Not until the next day had a large precipitate been separated, which exhibited under the microscope particularly large and well formed crystals.	4	266.9	4.0207	2.658	24.022	24.015	12.97	4.887
		13	252.4	4.0232	2.510	24.007			

4.85—4.86; the hydrogen-ion concentration is very slowly increasing with increasing ammonium sulphate concentration, owing to the fact that the solution of ammonium sulphate used contained a little surplus sulphuric acid.

Both from the figures of Table 39 and from their graphical representation it appears clearly that the crystallisation — espe-

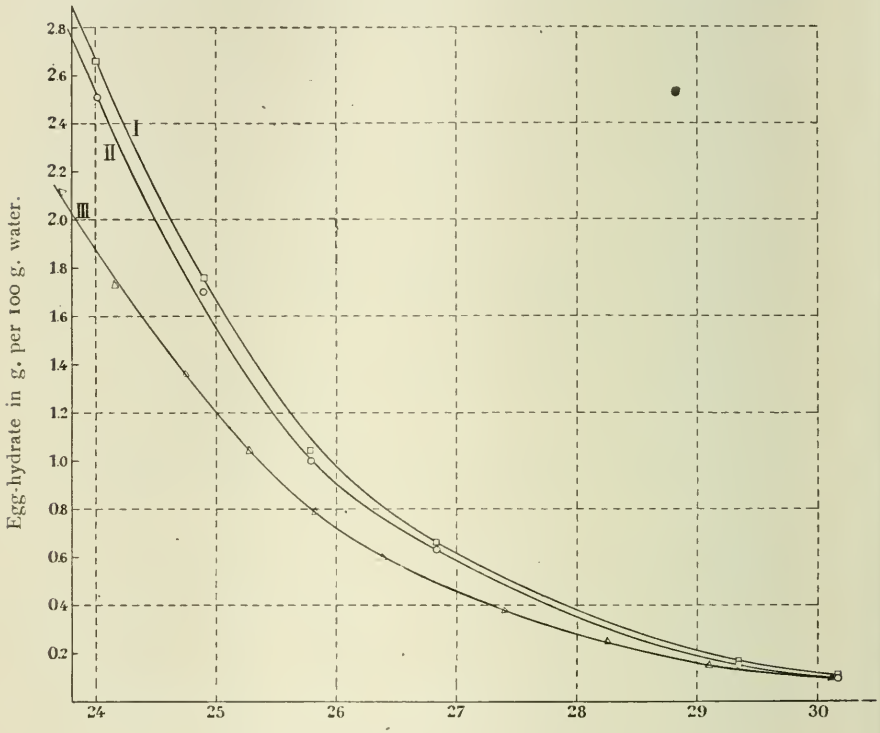

Fig. 24.

cially in the case of mixtures poor in ammonium sulphate — is not completed after 4 days' crystallisation, and in all likelihood the ultimate state of equilibrium is not reached even in the course of 13 days. It would therefore be of interest if it were possible to reach, or at least to approach, the state of equilibrium from the opposite side, that is, after abundant crystallisation of a mixture rich in ammonium sulphate, to dissolve some of the precipitate crystallised out by addition of water or a weak solution of ammonium sulphate. We have used such a method in the following series of experiments.

Series of experiments b. Also in this series, which comprises ten experiments executed in May 1916, the ratio of egg-albumin to water was in each mixture very nearly constant, namely about 7.2 g. egg-hydrate per 100 g. water, and the experiments were made at indoor temperature (about 18°). The experimental arrangement was as follows: —

To 2170 g. of a solution of egg-albumin of known composition we added 2157 g. of a concentrated solution of ammonium sulphate, also of known composition, and further 60 g. water and a few drops of inoculating material. The solution thus obtained, which very soon began to crystallise, contained per 100 g. water:

9.933 g. egg-hydrate and
28.348 g. ammonium sulphate.

The next day an abundant amount of crystals was found to have separated, and now 413 g. of the strong solution of ammonium sulphate were further added; the mixture was, while being repeatedly shaken, left to stand for some hours more for further crystallisation. The mixture of crystals and mother liquor thus obtained contained per 100 g. water

9.143 g. egg-hydrate and
30.128 g. ammonium sulphate.

From this mixture ten samples were weighed off in conical flasks closed with indiarubber stoppers; the weight of each sample was 400 g., and to each sample 80 cc. of a solution of ammonium sulphate was added very slowly, and while the mixture was well shaken. The concentration of the solution of ammonium sulphate was different in each experiment. For Experiment No. 1 we used the strongest concentration, and it was so adapted that the content of ammonium sulphate per 100 g. water in this experimental mixture was not altered by the addition of the 80 cc. For Experiment No. 10 we used 80 cc. pure water, and for all the interjacent experiments we used solutions of ammonium sulphate of intermediate, proper concentrations. During the first hour after addition the flasks were shaken repeatedly, and then one third of each mixture was filtered; another third was filtered the next day, and the remainder after the lapse of four days. The filtrations were executed exactly as in the series of experiments *a*, and the filtrates obtained were analysed; but determination of

TABLE 40. (Series of experiments *b*; May 1916).

All the experimental mixtures contained about 7.2 g. egg-hydrate per 100 g. water.

No. of exp.	Amount of egg-hydrate in 100 g. filtrate			Amount of ammonium sulphate in 100 g. filtrate after *Second* filtration	Thus the filtrate (*Second* filtration) contained per 100 g. water		Hydrogen-ion concentration of the filtrate (*Second* filtration)	
	First filtration (1 hour)	*Second* filtration (1 day)	*Third* filtration (4 days)		Egg-hydrate	Ammonium sulphate	$h \cdot 10^6$	pH
	g.	g.	g.	g.	g.	g.		
1	0.075	0.076	0.072	23.131	0.099	30.121	11.70	4.932
2	0.123	0.118	0.116	22.520	0.153	29.110	12.56	4.901
3	0.190	0.191	0.185	21.996	0.245	28.268	13.49	4.870
4	0.296	0.300	0.288	21.442	0.383	27.399	14.72	4.832
5	0.479	0.467	0.460	20.782	0.593	26.390	15.00	4.824
6	0.630	0.622	0.606	20.393	0.787	25.819	15.70	4.804
7	0.802	0.827	0.790	20.007	1.045	25.272	15.42	4.812
8	1.078	1.079	1.049	19.621	1.361	24.743	15.38	4.813
9	1.376	1.378	1.359	19.199	1.735	24.173	16.56	4.781
10	1.731	1.686	1.672	18.857	2.122	23.732	15.96	4.797

the amount of ammonia nitrogen was only made in the filtrates from the second filtration. The analytical results are set forth Table 40.

It appears from Table 40, second, third and fourth vertical columns, that the contents of egg-hydrate in the filtrates have certainly not undergone any material alteration from the first to the third filtration; yet it is throughout lower in the third filtrate than in the first and the second. This diminution of the egg-hydrate is commonly within the limits of the experimental errors; yet in a few cases it is so considerable that it must probably be attributed to the fact that we have not fully succeeded in avoiding the difficulty occasioned by the circumstance that an addition of water or weak ammonium sulphate solution to a mixture of crystals and mother liquor may cause a dissolution of the precipitate in the places where the drops are falling, a dissolution so abundant and fast that the amount of egg-albumin dissolved becomes greater than that corresponding with the state of equilibrium. On the whole, however, *the alterations in the contents of egg-hydrate between the first and the third filtrations must be said to be so small that we are here probably dealing with con-*

centrations which are surely not far from the real state of equilibrium of the systems.

Now as this series of experiments was made at the same temperature as the series *a*, and as the hydrogen ion concentration of the filtrates — as appears from the two last vertical columns of Table 40 — is of about the same magnitude[1]) as in the first series, we have in Fig. 24. besides the two curves (I and II) corresponding with the experiments *a*, represented those designated by *b* (Curve III), using as co-ordinates the figures in the sixth and seventh vertical columns of Table 40. If we compare the three curves of Fig. 24, and if we consider it a fact that Curve III indicates the state of equilibrium of the system, we arrive at the conclusion indicated above (p. 220), *viz.* that *the crystallisation in Experiments a even after the lapse of 13 days is not completely finished. and that the state of the system differs the more from the state of equilibrium as the concentration of ammonium sulphate decreases*[2]).

In the two series of experiments above described, the crystallisation of egg-hydrate has thus proved to take place so slowly, especially at low concentrations of ammonium sulphate,

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[1]) The mean value of p_H is 4.84; in contradistinction to what was found in Series *a*, the hydrogen-ion concentration in Series *b* is decreasing with increasing concentration of ammonium sulphate, which is due to the fact that the solution of ammonium sulphate used contained small amounts of surplus ammonia.

[2]) In the above comparison of the Curves I, II and III we have left out of consideration the circumstance that the hydrogen-ion concentration of the filtrates is not quite the same in all the experiments, and not even always the same in corresponding experiments of the two series. It appears from Tables 39 and 40, that at high concentrations of ammonium sulphate, the filtrates have a greater hydrogen-ion concentration in Series *a* than in *b*, while the case is the reverse at low concentrations of ammonium sulphate.

By means of the experiments described in Section C as to the influence of the hydrogen-ion concentration on the state of equilibrium, it is now possible to form an estimate about what the result of Experiments *b* would have been if the filtrates of this series had had the same hydrogen-ion concentration as the filtrates of Series *a* with the corresponding concentration of ammonium sulphate. If we use the experimental results thus corrected for the construction of Curve III, it appears that this curve in the case of the high concentrations of ammonium sulphate will be situated a little lower, and in the case of the low concentrations of ammonium sulphate a little higher, than shown in Fig. 24; but the displacements are so inconsiderable tha nothing is altered in the total picture given above of the experimental results.

that it is hardly possible, within a reasonable space of time, in this way to attain a real state of equilibrium. This conclusion is of course only correct under the assumption that the two series of experiments *a* and *b* are really comparable; in other words, that *in these two series, which were made at an interval of two years, and with two different solutions of egg-albumin, we had really to do with one and the same substance, so that the state of equilibrium in both cases ought to be the same.*

In support of the above explanation of our experimental results we may therefore state one more series of experiments, *c*, for which a third solution of egg-albumin was used. In this series we in some cases used direct crystallisation, as in Series *a*, while in other cases the method employed in Series *b* was adopted. Also in these experiments, described in greater detail below, and made with the same egg-albumin solution, it proved impossible to obtain a real state of equilibrium by direct crystallisation, whilst this was possible by the use of the method described under series *b*. We therefore see no reason to search for another explanation of the facts established in series *a* and *b* than that given above.

Series of experiments c. This series consisted of five experiments made in November 1914 at indoor temperature (abt. 18°) with mixtures of the solutions specified below: —

α) a *solution of egg-albumin* containing per 100 g. water

30.679 g. egg-hydrate and
16.767 g. ammonium sulphate;

β) a *strong solution of ammonium sulphate* containing per 100 g. water 33.090 g. ammonium sulphate, and

γ) a *weak solution of ammonium sulphate* containing per 100 g. water 16.120 g. ammonium sulphate.

Each of the five experimental mixtures contained 200 cc. of the egg-albumin solution and had besides the following composition:

No. 1: 300 cc. *strong* ammonium sulphate solution + 10 drops of inoculating material

No. 2: Just as No. 1; but after two days' crystallisation 100 cc. *weak* ammonium sulphate solution was added.

No. 3: Just as No. 1; but after two days' crystallisation 200 cc. *weak* ammonium sulphate solution was added.

No. 4: To the egg-albumin solution 100 cc. *weak* ammonium sulphate solution was added at once, and then 300 cc. *strong* ammonium sulphate solution and 10 drops of inoculating material.

No. 5: Just as No. 4; only we did not use 100, but 200 cc. of the *weak* ammonium sulphate solution.

Thus the mixtures No. 2 and No. 4 had the same composition, and so it is with No. 3 and No. 5; the experimental arrangement, however, was different, since the method used in Series of experiments *a* was employed for No. 4 and No. 5, while No. 2 and No. 3 were made as described under Series *b*. Finally, as to No. 1, this experiment had mainly for its object to ascertain how far the crystallisation had advanced in No. 2 and No. 3 after the lapse of two days before the addition of the weak ammonium sulphate solution. At proper times, samples were taken of the experimental mixtures, and the samples were filtered and analysed in the same manner as in Series *a* and *b*.

The first samples were taken out when all five mixtures had been left to stand two days for crystallisation, and the weak ammonium sulphate solution had been added in the experiments No. 2 and No. 3, these latter having been then left to stand one hour while being repeatedly shaken. Four hours later we again took samples of Nos. 2 and 3, but not of the other mixtures; so also on the next day, when we took out a third set of samples. With the following filtrations, however, samples of all the experimental mixtures were taken The analytical results are set forth in Table 41.

As to the figures given in Table 41, it is to be observed that all those in the seventh vertical column, corresponding with the contents of egg-hydrate in 100 g. filtrate after six weeks' crystallisation, are put in parentheses, being set forth for the sole purpose of elucidating a fact which we have sometimes met with in investigations of this kind.

It often happens, but not always, that the crystallised egg-albumin in mixtures of precipitate and mother liquor, if left to stand for a sufficiently long time at ordinary or higher temperature, is little by little redissolved, so that the amount of egg-hydrate of the mother liquor, instead of decreasing, is seen to increase. It is, we deem, beyond doubt that in such cases a bacterial process comes into play, by which crystallisable egg-albumin is transformed into a non-crystallisable, and more easily

TABLE 41. (Series of experiments *c*; November 1914).

Experimental mixture No. 1 contained about 11.5 g. egg-hydrate per 100 g. water.

Experimental mixtures Nos. 2 and 4 contained about 9.3 g. egg-hydrate per 100 g. water.

» » » 3 » 5 » » 7.9 » » » » » »

No. of Exp.	Amount of egg-hydrate in 100 g. filtrate.						Amount of ammonium sulphate in 100 g. filtrate after *Fourth* filtration	Thus the filtrate (*Fourth* filtration) contained per 100 g. water		Value of the *content of egg-hydrate* of the filtrate derived from Curves I and III (Fig. 24), respectively, and then corrected.	Hydrogen-ion concentration of the filtrate (*Fourth* filtration).	
	First filtration (2 days)	*Second* filtration (2 days + 5 hours)	*Third* filtration (2 days + 1 day)	*Fourth* filtration (2 days + 3 days)	*Fifth* filtration (2 days + 17 days)	*Sixth* filtration (2 days + 42 days)		Egg-hydrate	Ammonium sulphate		$h \cdot 10^9$	p_H
	g.	g.	g.	g.	g.	g.	g.	g.	g.	g.		
1	0.235			0.236	0.228	(0.236)	21.395	0.301	27.300	0.365	21.98	4.658
2	0.632	0.634	0.633	0.641	0.640	(0.664)	20.019	0.808	25.232	0.823	21.98	4.658
3	1.262	1.271	1.263	1.275	1.266	(1.313)	18.963	1.599	23.774	1.615	21.73	4.663
4	0.819			0.774	0.731	(0.743)	20.011	0.977	25.262	0.987	21.78	4.662
5	1.662			1.538	1.438	(1.431)	18.917	1.933	23.782	1.967	21.73	4.663

soluble compound. This transformation is probably — as adverted to in an earlier paper[1]) — the first stage of decomposition of the crystallisable egg-albumin, and it is probably of no essential importance, since the non crystallisable egg-albumin formed is coagulable just as the crystallisable, and the non-coagulable compounds resulting from the transformation are quite minimal in quantity.

Leaving out of consideration the figures in the seventh vertical column, it appears from Table 41 that, whilst in Experiments No. 2 and No. 3 — where a dissolution of already crystallised precipitate has taken place — we have arrived at a real state of equilibrium, this is not the case with the rest of the experiments, in which the direct method of crystallisation was used. It is true that this state of things does not manifest itself clearly in Experiment No. 1, where the concentration of ammonium sulphate is high; but in No. 4 and No. 5 the decrease of the egg-hydrate contained in the filtrates is quite unmistakable. It will be further seen that, although the quantities of ammonium sulphate (tenth vertical column) are the same in the experiments Nos. 2 and 4 and in Nos. 3 and 5, respectively, yet the contents of egg-hydrate are considerably smaller in No. 2 and No. 3 than in the corresponding experiments with direct crystallisation, in spite of the fact that the same solution of egg-albumin was used for all the experiments. Thus the state of affairs in this series corresponds, qualitatively, just with that existing in the first two series, and it will then be of interest to see if the accordance between the results of the three series of experiments is more extensive.

We shall first consider the experiments with direct crystallisation (Series *c*, Nos. 1, 4 and 5), which thus have to be compared with Series *a*. As the values of the egg-hydrate-contents set forth in the ninth and tenth columns of Table 41 relate to five days' crystallisation, it will be natural to use Curve I (Fig. 24) for the comparison, since this curve represents a crystallising time of four days. From Curve I we are able to ascertain how much egg-hydrate must be contained in the filtrate if the content of ammonium sulphate is equal to that found in Experiments Nos. 1, 4 or 5 (Series *c*), respectively, and if a direct comparison between the two series *a* and *c* is admissible. The figures thus derived

[1]) Comptes-rendus du Laboratoire de Carlsberg **12**, 37 (1916).

from Curve I will, however, turn out to be much higher than those stated in Table 41, ninth column. This is due to the fact that the two series of experiments are not directly comparable, because the hydrogen-ion concentration of Series *c* — as shown by the two last columns of Table 41 — is much greater (corresponding with $p_{H^\cdot}$ = about 4.66) than the hydrogen-ion concentration of the filtrates in Series *a* ($p_{H^\cdot}$ is here on an average equal to 4.85—4.86). Hence it follows, however, as will be proved in a subsequent section (see p. 240). that the amount of egg-hydrate in the filtrates of Experiments *c* must be smaller than that read from Curve I. Further, from the graphical representation of the dependence between the hydrogen-ion concentration and the amount of egg-hydrate remaining in the mother liquor after crystallisation (Fig. 26 p. 245), it can be estimated that the filtrate at a hydrogen-ion concentration corresponding with $p_{H^\cdot}$ = 4.66 contains only 68 % of the amount of egg-hydrate which, under otherwise equal conditions, is present in a filtrate having a hydrogen-ion concentration corresponding with $p_{H^\cdot}$ = 4.85. If therefore the figures read from Curve I are multiplied by 0.68, we get values represented by the amount of egg-hydrate which, within the limits of the experimental errors, must coincide with those stated in Table 41 ninth column. The figures thus deduced from Curve I and multiplied by 0.68 are set forth in the eleventh column of Table 41.

In quite the same way we have compared the experiments No. 2 and No. 3 (Series *c*) with Series *b*, and by means of quite similar considerations as above, the values of the contents of egg-hydrate read from Curve III are multiplied by 0.77, and the corrected values thus obtained are also set forth in the eleventh column of Table 41.

Now a comparison of the figures in the ninth and eleventh columns of Table 41 shows as nice accordance as was to be expected under the present conditions, and consequently we feel justified in drawing the conclusion that the *three different solutions of egg-albumin in the experiments here mentioned have behaved alike.*

B. The influence of Temperature on the Velocity of Crystallisation of Egg-albumin and on the State of Equilibrium between the Precipitate crystallised and the surrounding Mother Liquor.

Series of experiments d. Of the six experiments belonging to this series, which were made in September 1914, three (designated *R*, *S* and *T*) had the same concentration of ammonium sulphate (27.220 g. ammonium sulphate per 100 g. water), while the other three (designated *r*, *s* and *t*) had a somewhat lower concentration (26.007 g. ammonium sulphate per 100 g. water). The temperature of crystallisation was in the case of *R* and *r* 0° (the flasks standing in ice in an ice-safe), in *S* and *s* 12° (Panum's thermostat), in *T* and *t* 24° (Panum's thermostat). In every other respect the experimental arrangement was as described under Series of experiments *a*; for analyses of the filtrates samples were taken after the lapse of 2, 6 and 31 days, respectively.

The crystallisation took place but slowly in the experiments made at 0°. In *R* there was after a lapse of several hours but a small amount of precipitate, and it exhibited itself under the microscope as round amorphous matters mixed with a few bunches of crystals; it was not until the end of two days that the amorphous part of the precipitate had entirely disappeared, and an abundant precipitate consisting of fine needle-shaped but not very large crystals had formed. In *r* there was only after one day and night a fairly abundant crystallisation of normal appearance.

At 12°, and particularly at 24°, the crystallisation took place much more rapidly than at 0°, and the precipitate separated at once in the form of fine crystals.

In full accordance with what has been stated above, the analyses of the filtrates, the results of which are set forth in Table 42, proved that the *velocity of crystallisation is considerably lower at 0° than at 12° and 24°*. Further, the analytical results apparently go to show that *the state of equilibrium at 0° is essentially different from what it is at higher temperatures*, since even after the lapse of 31 days a considerably larger quantity of egg-hydrate is left in the solution in the experiments at 0° than in the other experiments. In order more closely to elucidate this, as well as

TABLE 42. (Series of experiments *d*; September 1914).
The experimental mixtures contained about 8.6 g. egg-hydrate per 100 g. water.

Mark of experiment.	Experimental temperature.	The filtrate contained per 100 g. water			
		Ammonium-sulphate.	Egg-hydrate		
			First filtration (2 days)	*Second* filtration (6 days)	*Third* filtration (31 days)
		g.	g.	g.	g.
R	0°	27.220	0.815	0.749	0.661
S	12°	,,	0.474	0.429	0.381
T	24°	,,	0.406	0.391	0.362
r	0°	26.007	1.712	1.534	1.395
s	12°	,,	0.956	0.877	0.781
t	24°	,,	0.805	0.744	0.714

several other questions, we have carried out the following series of experiments.

Series of experiments e. This series, which was made in February 1915, consisted of 12 experiments; the temperatures of crystallisation were 0°, 12°, 20° and 29°, three experiments being made at each temperature. They were made on the same principles as those followed in Series *c*. Thus equal amounts (100 cc.) of an egg-albumin solution of known composition were used for each of the three experiments. In the first experiments, 100 cc. of a "strong" solution of ammonium sulphate was added, and so also in the second; but after some days 100 cc. of a "weak" solution of ammonium sulphate was further added in this latter; in the third experiment, however, 100 cc. "weak" solution of ammonium sulphate was at once added, and immediately thereupon 100 cc. "strong".

The three experiments made at 0° were thus executed as follows:

No. 1: 100 cc. egg-albumin solution + 100 cc. "strong"
No. 2: ,, ,, ,, + ,, ,, + (after 5 days) 100 cc. "weak"
No. 3: ,, ,, ,, + 100 cc. "weak" + (at once) 100 cc. "strong".

In exactly the same way Nos. 4, 5 and 6 were made at 12°,

Nos. 7, 8 and 9 at 20°, and Nos. 10, 11 and 12 at 29°, only with this difference that in No. 8 and No. 11 the 100 cc. "weak" was not added after five, but after two days.

Nos. 1, 4, 7 and 10 had thus the same composition, and this could, on the basis of the known composition of the egg-albumin solution and of the "strong" and the "weak" solutions of ammonium sulphate, be calculated to be

$$\left.\begin{array}{l} 6.955 \text{ g. egg-hydrate} \\ \text{and } 28.233 \text{ g. ammonium sulphate} \end{array}\right\} \text{per } 100 \text{ g. water.}$$

In the rest of the experiments the composition was calculated to be

$$\left.\begin{array}{l} 4.467 \text{ g. egg-hydrate} \\ \text{and } 24.044 \text{ g. ammonium sulphate} \end{array}\right\} \text{per } 100 \text{ g. water.}$$

As for the rest, the experimental arrangement was the same as usual, and of course we took care that the solutions from which the experimental mixtures were prepared were previously heated or cooled to the temperature of the experiment. Finally, the proceeding was arranged in such a manner that we could take samples and effect the filtrations at the temperature in question.

All the experimental mixtures to which the "weak" solution of ammonium sulphate had not been added were more or less turbid; but no lumps of amorphous matter were found in any of them. The crystallisation commenced in all these mixtures in the course of half an hour; the samples kept at 20° and 29° crystallised the most rapidly and abundantly, the sample at 0° most slowly; but in all cases the precipitate thrown down appeared under the microscope to be distinctly crystalline.

On the other hand, the four mixtures which had received both "weak" and "strong" solution of ammonium sulphate crystallised but slowly, and this was more especially the case with the mixture which was left to stand at 0°. After the lapse of twenty-four hours, the crystallisation was abundant in No. 9 and No. 12 and fairly so in No. 6, while it was not until the end of several days that the crystallisation in No. 3 was well-marked, and even then far from abundant.

The analytical results of this series of experiments are set forth in Table 43, where the dates when the samples were taken

TABLE 43. (Series of exp

No. of Experiment	1	2	3	4	5
Composition of experimental mixture	"strong" without "weak"	"strong" + (after 5 days) "weak"	"weak" + (at once) "strong"	"strong" without "weak"	"strong" + (after 5 days "weak"
Amount of *ammonium sulphate* in g. per 100 g. water	28.233	24.044	24.044	28.233	24.044
Experimental temperature	0°	0°	0°	12°	12°
Amount of *egg-hydrate* (in g. per 100 g. water) of experimen					
Experimental mixtures before First filtration	6.955	4.467	4.467	6.955	4.467
First filtration. (after the lapse of — days)	0.379 (5 days)	2.293 (5 days + 1/2 hour)	4.410 (5 days)	0.229 (5 days)	1.489 (5 days + 1/2 hour)
Second filtration (after — days)	—	2.445 (5 days + 5 hours)	—	—	1.509 (5 days + 5 hours)
Third filtration (after — days)	—	2.556 (5 + 1 days)	—	—	1.499 (5 + 1 days
Fourth filtration (after — days)	0.342 (9 days)	2.566 (5 + 4 days)	4.379 (9 days)	0.217 (9 days)	1.489 (5 + 4 days
Temperature used after Fourth filtration	0° (as previously)	29° (previously 0°)	0° (as previously)	12° (as previously)	29° (previously 12°)
Fifth filtration (after — days)	0.336 (15 days)	1.789 (5 + 10 days)	4.347 (15 days)	—	1.689 (5 + 10 days
Sixth filtration (after — days)	0.318 (22 days)	1.800 (5 + 17 days)	4.253 (22 days)	0.209 (22 days)	1.680 (5 + 17 day
Temperature after Sixth filtration	Omitted	Omitted	20° (previously 0°)	Omitted	Omitted
Seventh filtration (after — days)	—	—	1.769 (27 days)	—	—

are also noted; in this connection it is only to be remarked that in the experiments (Nos. 2, 5, 8 and 11) to which, after a few days crystallisation, the "weak" solution of ammonium sulphate was added in order to reach the state of equilibrium by dissolution of the precipitate already separated, the samples were taken half

ents *c*; February 1915).

6	7	8	9	10	11	12
"weak" + (at once) "strong"	"strong" without "weak"	"strong" + (after 2 days) "weak"	"weak" + (at once) "strong"	"strong" without "weak"	"strong" + (after 2 days) "weak"	"weak" + (at once) "strong"
24.044	28.233	24.044	24.044	28.233	24.044	24.044
12°	20°	20°	20°	29°	29°	29°

xture before the first filtration, and of the different filtrates.

4.467	6.955	4.467	4.467	6.955	4.467	4.467
2.333 (5 days)	0.211 (2 days)	1.419 (2 days + 1/2 hour)	2.556 (2 days)	0.243 (2 days)	1.529 (2 days + 1/2 hour)	2.587 (2 days)
—	—	1.409 (2 days + 5 hours)	—	—	1.529 (2 days + 5 hours)	—
—	—	1.390 (2 + 1 days)	—	—	1.589 (2 + 1 days)	—
2.030	0.199	1.370	1.829	0.229	1.600	2.021
(9 days)	(6 days)	(2 + 4 days)	(6 days)	(6 days)	(2 + 4 days)	(6 days)
12° (as previously)	20° (as previously)	0° (previously 20°)	20° (as previously)	29° (as previously)	0° (previously 29°)	29° (as previously)
1.899	0.187	2.252	1.680	0.225	2.212	1.909
15 days)	(15 days)	(2 + 13 days)	(15 days)	(15 days)	(2 + 13 days)	(15 days)
1.810	0.181	2.202	1.589	0.224	2.131	1.860
22 days)	(22 days)	(2 + 20 days)	(22 days)	(22 days)	(2 + 20 days)	(22 days)
0° previously 12°)	Omitted	Omitted	0° (previously 20°)	Omitted	Omitted	0° (previously 29°)
2.181	—	—	1.990	—	—	2.071
27 days)			(27 days)			(27 days)

an hour (First filtration), five hours (Second filtration), twenty-four hours (Third filtration) and four days (Fourth filtration) after the addition of the "weak" solution of ammonium sulphate.

Common to all the experiments is the fact that the determination of the amount of ammonium nitrogen was only made

in one of the filtrates (Fourth filtration). These determinations showed per 100 g. water in

No. 1:	28.211 g.	ammonium	sulphate
„ 4:	28.227 „	„	„
„ 7:	28.265 „	„	„
„ 10:	28.230 „	„	„
Average:	28.233 g.	ammonium	sulphate

No. 2:	24.027 g.	ammonium	sulphate
„ 3:	24.070 „	„	„
„ 5:	24.052 „	„	„
„ 6:	24.055 „	„	„
„ 8:	24.033 „	„	„
„ 9:	24.005 „	„	„
„ 11:	24.054 „	„	„
„ 12:	24.052 „	„	„
Average:	24.044 g.	ammonium	sulphate

It appears from these figures, that the concentration of ammonium sulphate is practically the same within either of the two groups of mixtures, respectively, the differences from the mean values being so small as to be attributable to analytical errors. Therefore we have in Table 43 only stated the mean values.

Finally it is to be mentioned that after taking samples for the fourth filtration we altered the temperature in several experiments. Our intention in so doing was to investigate the possibility of producing alterations in the state of equilibrium through alterations of temperature. Further information is given in the last five horizontal columns of Table 43.

Let us first consider that part of Table 43 which represents the analytical results of the filtrates obtained by the first four filtrations. These results are graphically represented by the four systems of curves in Fig. 25, where the time of crystallisation in days is plotted as abscissa against the content of egg-hydrate of the filtrate in g. per 100 g. water as ordinate.

If we consider more closely one of the systems of curves, *e. g.* the system corresponding with the temperature 20°, comprehending the experiments Nos. 7, 8 and 9, the course of the curves corresponding with Nos. 7 and 9 will be easily understood. Already from the figures of Table 43, but still more clearly from

the curves, it is seen that in No. 7 the crystallisation is nearly finished in the course of two days, while this is far from being the case in No. 9, where the concentration of ammonium sulphate is much weaker. As for Experiment No. 8, it is to be supposed that during the first two days the crystallisation takes a course quite similar to that observed in Experiment No. 7; but now the

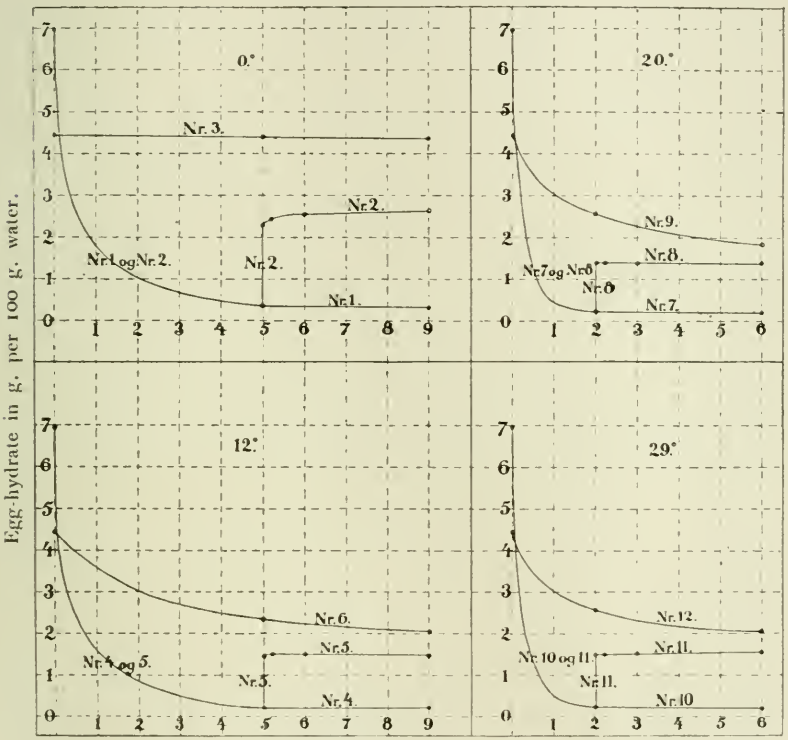

Fig. 25.

"weak" solution of ammonium sulphate is added to No. 8, and it appears clearly from the curve how the content of egg-hydrate of the solution is thereby increased and already at the first taking of samples ($^1/_2$ hour later) reaches its maximum, which then remains fairly constant, corresponding with the actual state of equilibrium. It further appears from the figure that the curve No. 9, corresponding to the experiment with direct crystallisation, certainly approaches the curve No. 8, but that there is constantly a marked distance between these two curves, a circumstance

which means that the state of equilibrium has not been reached in Experiment No. 9 even after six days' crystallisation.

The other three systems of curves in Fig. 25 are to be interpreted in quite an analogous manner. From these curves, as well as from the figures of Table 43, it appears that the *velocity of crystallisation is much lower at 0° than at the other temperatures:* this fact is seen especially clearly in Experiment No. 3, where the curve of crystallisation is nearly parallel to the axis of abscissa. As for the other three temperatures, the difference in the velocity of crystallisation is far less marked; a closer examination of the figures shows, however, that the *velocity of crystallisation is, in general, the higher as the temperature increases.* The difference between the quantity of egg-hydrate found in the fourth filtrate of No. 3 (direct crystallisation) and the corresponding value for No. 2 (state of equilibrium) gives at any rate a fairly correct measure of the amount of egg-hydrate which at the experimental temperature 0° still exists in the solution beyond the amount corresponding with the state of equilbrium, and an analogous view may of course be taken in regard to the other experimental temperatures. This difference is seen to decrease as the temperature increases:

At	0°	the	difference	is 1.813
„	12°	„	„	„ 0.541
„	20°	„	„	„ 0.459
„	29°	„	„	„ 0.421

It is an obvious conclusion that the reason is to be sought in the circumstance that the velocity of crystallisation increases with increasing temperature. This interpretation also agrees with the immediate impression received on letting one and the same mixture crystallise at different temperatures.

In regard to the state of equilibrium of the systems, the experimental temperature 0° is likewise found to differ widely from the other temperatures employed. While the system examined by us, which contained 24.044 g. ammonium sulphate per 100 g. water, in the state of equilibrium per 100 g. water exhibits about 1.6 g. egg-hydrate at 29°, about 1.4 g. at 20° and about 1.5 g. at 12°, it is at 0° found to contain more than 2 g. Besides, the figures go to show that *between 12° and 29° — perhaps about 20° — there exists an optimum temperature at which the smallest*

amount of egg-hydrate is present in the solution in the state of equilibrium and under otherwise the same conditions. The discrepancies observed are certainly rather inconsiderable, but we advert to them because experiments undertaken with other objects in view have led to results pointing in the same direction[1]). In this connection we may further draw attention to the fact that the experiments for determination of the factor r described in the preceding paper[2]) do not lend any countenance to the assumption that at higher temperatures an egg-hydrate of another composition would crystallise out than that separated at lower temperatures; but on the other hand we must remember that the highest temperature used in the determination of r was 24^0.

As mentioned above, some of the experimental mixtures were placed, after we had taken samples for the fourth filtration, at other temperatures than those at which these mixtures had been hitherto left to stand. Our purpose was to ascertain if we were in presence of a general state of equilibrium dependent on the temperature and susceptible of being displaced in one or another direction by proper alterations of the temperature. The experimental results showed conclusively that this was really the case. Both the experimental arrangement and the analytical results are set forth in the last five horizontal columns of Table 43.

Thus it appears from the table that experimental mixture No. 2, which was in the state of equilibrium at 0^0 and which at the end of nine days was transferred to 29^0, thereafter continued crystallising, and the case was the same with No. 3, which after direct crystallisation for 22 days was transposed from 0^0 to 20^0. That the opposite effect, a dissolution of crystallised matter, can be produced by displacement from 20^0 or 29^0 to 0^0, appears from the experiments No. 8 and No. 11 (state of equilibrium) and also from the experiments No. 9 and No. 12 (direct crystallisation). Finally, Experiment No. 5 shows that a displacement to 29^0 of a system in equilibrium at 12^0 will give rise to a slight dissolution of egg-hydrate already crystallised out; this fact is in full accordance with that mentioned above: that the concentration of egg-hydrate is greater at 29^0 than at 12^0, if the system is in equilibrium at the temperature concerned. *Qualitatively, the*

[1]) Compare H. Chick and C. J. Martin: Biochem. Journ. **7**, 389 (1913).
[2]) Comptes-rendus du Laboratoire de Carlsberg **12**, 170 (1917).

mixtures of crystals of egg-albumin and surrounding mother liquor here examined thus behave just as systems in a state of equilibrium dependent on the temperature. It is, however, to be observed that the quantitative accordance between the concentrations of egg-hydrate in the different experiments which in different ways were brought under the same conditions is not so good as desirable. An examination of the last columns of Table 43 shows that we have here to deal with differences somewhat greater than our ordinary experimental errors. The reason is probably that the dissolution, and especially the crystallisation, of the egg-hydrate take place so slowly, especially at low temperatures, that it is hardly possible within reasonable spaces of time to reach a complete and absolute state of equilibrium.

C. Influence of the Hydrogen-ion concentration on the Velocity of Crystallisation of Egg-albumin and on the State of Equilibrium between the Precipitate crystallised and the surrounding Mother Liquor.

Series of experiments f. This series, made in August 1914 at indoor temperature, consisted of 18 experiments. The concentration of egg-hydrate was before the crystallisation nearly the same in all the experiments, while the concentration of ammonium sulphate was somewhat lower in the first nine than in the last nine experiments; in fact, the experimental mixtures No. 1 to No. 9 per 100 g. water were found to contain:

25.947 g. ammonium sulphate
and 8.594 g. egg-hydrate;

and the mixtures No. 10 to No. 18:

27.121 g. ammonium sulphate
and 8.642 g. egg-hydrate.

Within each of the two groups of experiments the hydrogen-ion concentration varied from $p_{H'}$ = about 3.9 to $p_{H'}$ = about 5.3.

For the preparation of these mixtures we used a solution of egg-albumin and a strong solution of ammonium sulphate, both of known composition, and further, three solutions prepared from the latter and all containing the same amount of ammonium sulphate, but of which one (designated "acid") had received an ad-

mixture of diluted sulphuric acid, the second (designated "alkaline") of diluted ammonia, and the third (designated "neutral") of water.

We first prepared two stock solutions, one for each of the two groups of experiments, and so that the solution (designated "weak") intended for the experiments Nos. 1 to 9 was prepared from

1000 cc. egg-albumin solution
430 „ water
and 570 „ ammonium sulphate solution,

while the stock solution (designated "strong") for Nos. 10 to 18 consisted of

1000 cc. egg-albumin solution
395 „ water
and 605 „ ammonium sulphate solution.

Both these stock solutions remained completely limpid during the mixing and subsequent measuring for the single experiments; but after the lapse of a few hours the remainder of "strong" began to crystallise, and the next day the case was the same with the remainder of "weak".

The experimental mixtures were prepared by mixing exactly measured amounts of the stock solution with small amounts of "acid", "neutral" and "alkaline" adapted to the hydrogen-ion concentration wanted, in such a manner that we always used 3 cc. of the three latter solutions in all for each 50 cc. stock solution. The composition of the first nine mixtures was as follows: —

No. of exp.	Stock sol. "weak"	"acid"	"neutral"	"alkaline"
1	150 cc.	9.0 cc.	0.0 cc.	0 cc.
2	150 „	7.5 „	1.5 „	0 „
3	250 „	10.0 „	5.0 „	0 „
4	250 „	7.5 „	7.5 „	0 „
5	250 „	5.5 „	9.5 „	0 „
6	250 „	3.5 „	11.5 „	0 „
7	250 „	1.5 „	13.5 „	0 „
8	250 „	0.0 „	15.0 „	0 „
9	150 „	0.0 „	7.5 „	1.5 „

The composition of the nine other mixtures was quite analogous to those just mentioned, so that No. 10 corresponded with No. 1, No. 11 with No. 2, *etc.*, only with the difference that stock solution "strong" was used instead of "weak".

As for the crystallisation of these mixtures, the following observations must be premised: To the measured stock solution

the fixed amount of "neutral" was first added, the precipitate hereby separated could in the case of all the solutions be redissolved by shaking, and so it was after the subsequent addition of "alkaline" to No. 9 and No. 18 and of "acid" to Nos. 5, 6, 7, 15 and 16. In all these mixtures, the crystallisation commenced in the course of $^1/_4$—$^1/_2$ hour and was seen to take the normal course. On the other hand, Nos. 1, 2, 3, 4, 10, 11, 12, 13 and 14, that is, all the most acid mixtures, gave on addition of "acid" so voluminous a precipitate that it was not possible even by continued careful shaking to redissolve it; in the case of the most acid samples, moreover, the precipitate was of a somewhat sticky consistency, which caused a part of the precipitate to adhere to the bottom and sides of the flask. This precipitate, which was present in Nos. 1, 2, 3, 10 and 11, did not undergo any alteration during the time when the mixtures were left to stand, whereas the suspended, amorphous precipitate little by little grew crystalline, just as, even from the most acid mixtures, a pronounced crystalline precipitate was thrown down. Concerning the appearance of the precipitate under the microscope, the reader is referred to Table 44, second vertical column.

The taking of samples with subsequent analysis of the filtrate was made in the usual manner after the lapse of 2, 5 and 21 days. The amount of ammonia nitrogen was estimated only in the filtrates from the second set of samples (after the lapse of five days) and gave the following results:

In the different filtrates we found g. ammonium sulphate per 100 g. water:

No. 1: (26.032) g.	No. 10: (27.481) g.
„ 2: (26.013) „	„ 11: (26.978) „
„ 3: 25.953 „	„ 12: 27.130 „
„ 4: 25.960 „	„ 13: 27.130 „
„ 5· 25.933 „	„ 14: 27.147 „
„ 6: 25.933 „	„ 15: 27.120 „
„ 7: 25.929 „	„ 16: 27.096 „
„ 8: 25.966 „	„ 17: 27.102 „
„ 9[1]): 25.954 „	„ 18[1]): 27.125 „
Average: 25.947 g.	Average: 27.121 g.

[1]) In calculating the amount of ammonium sulphate of No. 9 and No. 18 it is taken into account that "alkaline" has been added to these two mixtures; the amount of surplus ammonia in the solution "alkaline" is not included in the above stated quantities of ammonium sulphate.

From the above table it appears that the two most acid mixtures in each group show an amount of ammonium sulphate a little different from that of the other mixtures in the same group; this is not to be wondered at, since precisely these experiments gave, as already mentioned, an abundant separation of resinous precipitate, which has probably another composition than the crystallised egg-albumin. If we do not take these experiments into consideration, the accordance between the values found for the contents of ammonium sulphate is excellent, and we have therefore, in Table 44, noted only the mean values of the determinations, just as we have used these mean values in calculating the amounts of egg-hydrate of the filtrates.

The hydrogen-ion concentration of the filtrates was measured both after 5 days' and 21 days' crystallisation; the accordance between the two series of measurements was in most cases satisfactory, and we have in Table 44 noted only the mean values of the measurements.

In Table 44, the experimental results are set forth in the usual way, and in Fig. 26, which graphically represents the most important of these results, the content of egg-hydrate (in mg. per 100 g. water) in the filtrate is used as ordinate, while the p_H-value of the filtrate is taken as abscissa.

The topmost bunch of curves on Fig. 26 corresponds with the experiments at the lower concentration of ammonium sulphate (Curves I, II and III answering to the time of crystallisation 2, 5 and 21 days, respectively), while the lower bunch represents the experiments with the higher concentration of ammonium sulphate (Curves IV, V and VI answering to the time of crystallisation 2, 5 and 21 days, respectively). The results of the most acid mixtures which, as repeatedly mentioned, did not crystallise in the normal way, are not shown in Fig. 26, and the same applies to the two least acid mixtures, which — owing to the scale adopted — could not find room on the figure. *Thus, Fig. 26 represents only the central range of the hydrogen-ion concentration of egg-albumin crystallisation, and it appears clearly both from Fig. 26 and Table 44 that within this range there exists an optimal hydrogen-ion concentration at which the crystallisation under otherwise equal conditions advances farthest. This optimal hydrogen-ion concentration corresponding with p_H = about 4.58 is very nearly equal in both the groups of experiments here mentioned, and*

TABLE 44 (Series of experiments *f*; August 1914).

No. of Experiment.	Appearance of the precipitate under the microscope.	The filtrate contained			Hydrogen-ion concentration of filtrate (5 days and 21 days).	
		First filtration (2 days) g. egg-hydrate per 100 g. water	*Second* filtration (5 days) g. egg-hydrate per 100 g. water	*Third* filtration (21 days) g. egg-hydrate per 100 g. water	$h \times 10^6$	p_H
The experimental mixtures contained 25.947 g. ammonium sulphate and 8.594 g. egg-hydrate per 100 g. water.						
1	After the lapse of one day the precipitate adhered to the flask in form of a tough mass, which under the microscope proved to be completely amorphous. This sticky mass did not alter its character in the course of time; but in the liquid another precipitate was formed, which was easily to be suspended and voluminous, and under the microscope appeared as distinct crystals (No. 1 and No. 2 quite small crystals, No. 3 somewhat larger crystals).	3.214	0.977	0.797	115.0	3.(
2		3.207	1.922	0.860	81.3	4.(
3		3.814	0.711	0.712	63.1	4.2
4	Very small but well-shaped, needle-formed crystals, which only in few cases were collected in bunches or heaps.	0.550	0.512	0.492	42.7	4.3
5		0.445	0.416	0.389	31.6	4.5
6	Needle-formed, rather large crystals collected in bunches or heaps.	0.459	0.428	0.402	22.4	4.6
7		0.667	0.639	0.599	14.1	4.8
8	Large, fine, needle-formed crystals.	1.096	1.032	0.982	9.55	5.0
9	Long, well-shaped, prismatic crystals.	3.684	3.217	2.914	5.01	5.3
The experimental mixtures contained 27.121 g. ammonium sulphate and 8.642 g. egg-hydrate per 100 g. water.						
10	The appearance was exactly as described in the case of Nos. 1 and 2.	1.315	1.249	0.333	120.2	3.9
11		1.736	1.447	0.468	87.1	4.0
12	The precipitate consisted of micro-crystalline and resinous masses, which gradually grew distinctly crystalline.	0.354	0.330	0.317	60.3	4.2
13	Just as Nos. 4 and 5.	0.269	0.252	0.245	39.8	4.4
14		0.232	0.215	0.197	28.8	4.5
15	Just as Nos. 6 and 7.	0.229	0.222	0.202	22.9	4.6
16		0.335	0.326	0.301	14.1	4.8
17	Large, needle-formed crystals, often collected in bunches.	0.584	0.555	0.528	10.0	5.0
18	Large, needle-formed crystals, mixed with long well-shaped prisms.	1.812	1.693	1.595	6.03	5.2

the situation of the optimum is not appreciably displaced during the progressive crystallisation.

It must be remembered that the experiments here mentioned are direct crystallisation experiments, in which we cannot there-

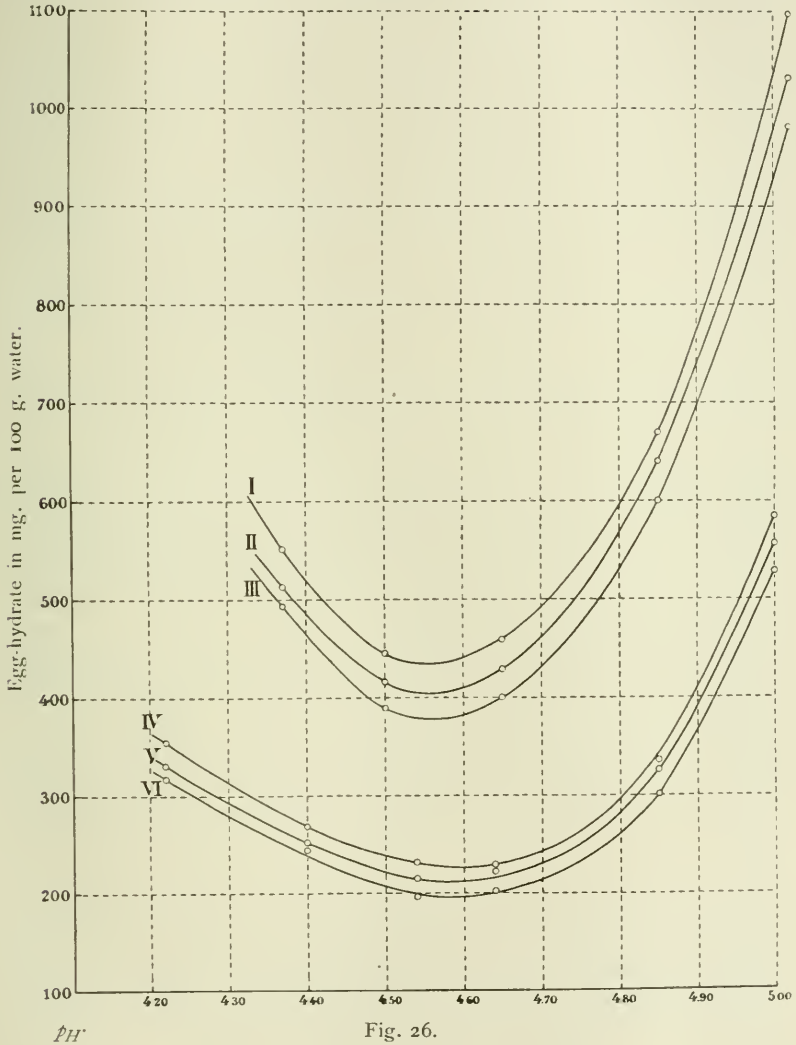

Fig. 26.

fore expect to reach the real state of equilibrium even after 21 days' crystellisation; but from the experience acquired up to now (see for instance Section A) we have every reason to expect that the curves corresponding with the real states of equilibrium must be of quite the same appearance as the curves in Fig. 26, though

situated a little lower than these. Consequently, as to the real equilibrium, the optimum will probably be situated at p_{H^+} = about 4.58. In this connection it may be added that in some preliminary experiments, made in February 1913 at a temperature of abt. 5°, as to the significance of the hydrogen-ion concentration for the velocity of crystallisation of egg-albumin, we found that even at this rather low temperature the albumin crystallised under otherwise the same conditions, most completely at hydrogen-ion concentrations corresponding with p_{H^+} = 4.5—4.6.

On both sides of the optimum the crystallisation will of course be less complete than it is at the very optimal hydrogen-ion concentration; but the conditions are somewhat different according as we move towards the alkaline or towards the acid side. Fig. 26 gives a distinct impression of how precipitously the curves ascend with increasing p_{H^+}, and from the values stated in Table 44 for the amount of egg-hydrate in the filtrates from Experiments Nos. 8 and 9 and Nos. 17 and 18, respectively, it further appears that an exceedingly great increase of egg-hydrate will answer to a slight increase of p_{H^+}. *A decrease of the hydrogen-ion concentration through an addition of ammonia has thus, for the question here discussed, only the influence that the amount of egg-albumin crystallised is diminished, whereas the appearence and character of this precipitate are not altered.*

The case is quite different if the hydrogen-ion concentration be increased, by addition of sulphuric acid, beyond the optimum. A slight increase of the hydrogen-ion concentration will only give rise to a comparatively slight increase of the amount of egg-hydrate in the mother liquor: but a greater increase of the hydrogen-ion concentration gives not only a greater increase of the egg-hydrate of the mother liquor, but also a thorough alteration of the appearance and character of the precipitate separated; we are here probably in presence of a precipitation of a compound richer in sulphuric acid than the common crystallised egg-albumin A more exact examination of Table 44 will further show that the amounts of egg-hydrate in the filtrates are generally higher in Experiment No. 2 than in No. 1, and in No. 11 than in No. 10. If this is more than an accident, it suggests that the precipitation — there is hardly question of crystallisation — will at still higher concentrations again increase, which would agree perfectly with the well-known phenomenon that the precipitating action of such salts

as ammonium sulphate is increased by addition of acids. From Table 44 it is further seen that the increase of the content of egg-hydrate of the filtrate with increasing hydrogen-ion concentration is less pronounced at higher than at lower concentrations of ammonium sulphate; at any rate this appears clearly at the third filtration, where the conditions may probably be supposed to approach the state of equilibrium. It is possible, therefore, that at sufficiently high concentrations of ammonium sulphate we may not observe any increase of the egg-hydrate of the filtrate with increasing hydrogen-ion concentration, but on the contrary a gradual decrease, as H. Chick and C. J. Martin[1]) have found by precipitation — not crystallisation — of egg-albumin at different hydrogen-ion concentrations and in presence of abundant quantities of ammonium sulphate.

In order to elucidate a circumstance which we have already adverted to in Section A of this paper (see p. 227), we may add that after the completion of the experiments above described the remainders of the experimental mixtures were left to stand for a few months more in the flasks at indoor temperature. During this time several samples did not suffer any visible alteration, and the analyses then effected showed quite a similar amount of egg-hydrate in the filtrates to that found after the third filtration; this was the case especially of the least acid of the mixtures rich in ammonium sulphate (Nos. 14, 15, 16, 17 and 18). As to the other mixtures, and especially Nos. 2, 3, 4, 8, 10, 11, 12 and 13, the crystallisable egg-albumin was found to have undergone a bacterial transformation into non-crystallisable matters. The quantity of precipitate separated had in these samples been more or less diminished; in Experiment No. 2 the precipitate was even completely dissolved. The egg-hydrate of the solution had of course increased correspondingly, the transformation not having advanced sufficiently to produce more than quite minimal amounts of non-coagulable compounds.

D. The Influence of Protein Concentration on the Velocity of Crystallisation of Egg-albumin, and on the State of Equilibrium between the Precipitate crystallised and the surrounding Mother Liquor.

Already in the introduction to this paper (p. 216) it is mentioned that proteins under otherwise equal conditions are generally supposed to be salted out the more completely as the

[1]) Biochem. Journ. **7**, 386 (1913).

TABLE 45.

Experiment of Chick and Martin

(*loc. cit.*, p. 385, Table III).

Ratio salt/water constant = $^{31}/_{100}$.

Concentration of protein varying.

Albumin g.	Water g.	Salt g.	G. albumin in 100 g. total mixture.	G. albumin to 31 g. Salt and 100 g. H_2O.	G. albumin in 100 g. filtrate.	G. albumin precipitated from 100 g. total mixture.	Protein precipitated %
1.90	56.92	17.65	2.481	3.33	1.130	1.351	54.4
1.90	36.92	11.44	3.775	5.14	1.115	2.660	70.4
3.79	50.85	15.76	5.383	7.45	1.159	4.224	78.5
7.59	53.69	16.64	9.738	14.13	0.935	8.803	90.4
4.74	17.31	5.36	17.306	27.40	0.772	16.534	95.5

starting protein concentration of the solutions is increased, and as an example of the investigations concerning this question we quoted H. Chick and C. J. Martin's[1]) work on the precipitation of egg-albumin by means of ammonium sulphate. In the introduction we have also pointed out how the experimental results obtained by Chick and Martin may, in our opinion, be accounted for, and we shall now, referring to the details of the experiments, try to prove the correctness of this interpretation.

Chick and Martin have made a series of precipitations of purified egg-albumin by means of ammonium sulphate; the concentration of albumin was different in each experiment, while the ratio ammonium sulphate to water was the same, namely 31 : 100. Table 45 reproduces Chick and Martin's numerical data concerning this series of experiments.

As the said authors determine the contents of albumin of the filtrates[2]) by heat coagulation and subsequent weighing of the filtered, washed and dried coagulum, we take it as a fact that also the quantities stated in Table 45 refer to the weight of albumin as determined in the above mentioned manner. Chick and Martin now evidently suppose that the egg-albumin, by precipitation with ammonium sulphate, is separated in the same

[1]) Biochem. Journ. **7**, 380 (1913).

[2]) *loc. cit.*, p. 383.

composition as by heat coagulation, that is, in the anhydrous state, because it is only under this assumption that the ratio of ammonium sulphate to water will be the same in all the filtrates and equal to the original ratio 31 : 100. From the description of the experiments, it does not appear whether determinations of the amounts of ammonia nitrogen of the filtrates were effected or not, and therefore we are inclined to believe that such determinations were not made. We consider it highly improbable that the egg-albumin, by precipitation with ammonium sulphate, should be separated in water-free condition. There will surely not be any essential difference between precipitation and crystallisation of egg-albumin by means of ammonium sulphate, and therefore we consider that the albumin is probably precipitated with a similar amount of water to that with which it crystallises. But, if this assumption be correct, it evidently follows that Chick and Martin's interpretation of their experimental results is incorrect, as in this case, water will, by the precipitation, be removed from the solution, so that the concentration of ammonium sulphate increases, and to so much greater an extent as more egg-albumin is precipitated, or, in other words, the greater the starting concentration of the albumin.

We have undertaken a re-calculation of Chick and Martin's experiments, presupposing that the egg-albumin separated by precipitation, just as the crystallised albumin, contains 0.22 g. water per g water-free egg-albumin[1]). We have no guarantee that just this amount of water answers to the real fact, but we have no doubt that this mode of calculation is more adequate than that used by Chick and Martin. The results of the re-calculation are set forth in Table 46.

It appears from Table 46 that, assuming the method of calculation adopted to be correct, the filtrates in the experiments of Chick and Martin will have the higher a concentration of ammonium sulphate and the lower a concentration of egg-hydrate, the greater the amount of egg-hydrate precipitated[2]). If we represent graphically the dependence between the salt and the egg-hydrate concentrations of the filtrates, we obtain

[1]) Comptes-rendus du Laboratoire de Carlsberg **12**, 182 (1917).

[2]) Experiment No. 3 does not tally with the other experiments; but it appears already from Chick and Martin's figures (Table 45, sixth vertical column) that an experimental error has been committed.

TABEL 46.

Re-calculation of Chick and Martin's experiment.

Egg-hydrate Albumin + Water	Water	Salt	In the original mixture is thus found per 100 g. water:		Egg-hydrate in 100 g. filtrate	Water + Salt in 100 g. filtrate	Thus the filtrate contained per 100 g. water:	
			Egg-hydrate	Salt			Egg-hydrate	Salt
g.	g.	g.	g.	g.	g.	g.	g.	g.
1.90 + 0.42 = 2.32	56.92 ÷ 0.42 = 56.50	17.65	4.11	31.24	1.130 × 1.22 = 1.379	98.621	1.835	31.24
1.90 + 0.42 = 2.32	36.92 ÷ 0.42 = 36.50	11.44	6.36	31.34	1.115 » = 1.360	98.640	1.811	31.34
3.79 + 0.83 = 4.62	50.85 ÷ 0.83 = 50.02	15.76	9.24	31.51	1.159 » = 1.414	98.586	1.886	31.51
7.59 + 1.67 = 9.26	53.69 ÷ 1.67 = 52.02	16.64	17.80	31.99	0.935 » = 1.141	98.859	1.523	31.99
4.74 + 1.04 = 5.78	17.31 ÷ 1.04 = 16.27	5.36	35.53	32.94	0.772 » = 0.942	99.058	1.264	32.94

Curve II in Fig. 27, which is drawn without regard to experiment No. 3 (see the note p. 249). Besides this curve, Fig. 27 contains one marked I, which represents the dependence between the salt and egg-hydrate concentrations of the filtrates in another series of experiments made by Chick and Martin[1]), just in order to investigate the dependence here mentioned. We have re-calculated this series in quite the same manner as that above mentioned; the re-calculation is, however, here of much less consequence, as all the experiments had the same starting concentration of albumin, which was, moreover, quite minimal, *viz.* 1.3 g. water-free albumin per 100 g. water. From Fig. 27 it appears that the two curves are quite parallel, and we draw from this the conclusion that Curve II, like Curve I, gives a picture of the dependence between the salt concentration and the egg-hydrate concentration, but has nothing to do with the starting protein concentration of the experimental mixtures. The fact that the two curves do not coincide must be due to the circumstance that the two series of experiments represented by the curves were not made under the same conditions, for instance the same hydrogen-ion concentration. That such a difference between the two series really exists, appears clearly from Chick and Martin's numerical results. In the series corresponding with Curve I, for instance, the experimental mixture containing per 100 g. water 31 g. salt and 1.3 g. albumin contains only 0.733 g. albumin in 100 g. filtrate, whereas it appears from Table 45 that in the corresponding series, where all the mixtures contain 31 g. salt per 100 g. water, 100 g. filtrate always contain more than 0.733 g. albumin, and at so low a starting concentration of albumin as 1.3 g. per 100 g. water, the amount of albumin in 100 g. filtrate must be superior to 1.130 g. (Table 45, first experiment).

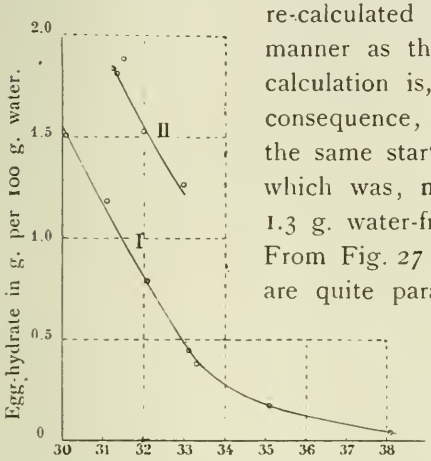

Fig. 27.

[1]) *loc. cit.*, p. 383, Table II.

We think therefore, that the results obtained by Chick and Martin in the experiments here described, prove nothing as to any influence exerted by the starting protein concentration on the composition of the filtrate, but may be easily accounted for as an effect of the different salt concentrations of the filtrates. The question then arises: *How will the protein concentration of the filtrate depend on the starting protein concentration, if we adapt the experimental arrangement in such a way that the concentration of ammonium sulphate is really the same in all the filtrates?* Already in the introduction to this paper we have shown that according to Gibbs' phase rule the starting protein concentration does not influence the protein concentration of the filtrate, and we have tried to prove that this is really the fact, by means of the following two series of experiments.

Series of experiments g. This series, made in February 1915 at indoor temperature (18°), consisted of five experiments. While all the experimental mixtures had very nearly the same concentration of ammonium sulphate (on an average 26.658 g. ammonium sulphate per 100 g. water), the starting concentration of egg-hydrate varied in the five experiments approximately as 6 : 4 : 3 : 2 : 1, the contents of egg-hydrate per 100 g. water amounting to 14.140 g. (No. 1), 9.419 g. (No. 2), 7.058 g. (No. 3), 4.703 g. (No. 4) and 2.350 g. (No. 5), respectively.

These mixtures were prepared with a solution of egg-albumin, a "weak" and a "strong" solution of ammonium sulphate, all solutions of known composition. We now measured

for	No. 1:	180 cc.	egg-albumin	solution
	„ 2:	120 „	„	„
	„ 3:	90 „	„	„
	„ 4:	60 „	„	„
	„ 5:	30 „	„	„

After weighing the measured egg-albumin solutions, we calculated how much of the "weak" solution of ammonium sulphate and how much water had to be added to the last four solutions for these to contain each the same amount of water and ammonium sulphate as No. 1, so that solely the contents of egg-hydrate would be different. The quantities calculated being then added, each solution was precipitated by 100 cc. "strong" solution of

ammonium sulphate, and finally each mixture received five drops of inoculating material.

After being mixed with the "strong" solution of ammonium sulphate all the solutions could be completely freed from amorphous precipitate by shaking. No. 1 was, however, highly, No. 2 slightly opalescent; the rest were limpid.

No. 1 crystallised distinctly in the course of $^1/_4$ hour, and in $1^1/_2$ hours it formed a mass of pappy consistency.

No. 2 and No. 3 crystallised in the course of $^1/_2$ to $^3/_4$ hour, and after 3 hours these two mixtures were also found to form nearly pappy masses.

No. 4 crystallised more slowly, and not until the course of 6 hours an abundant precipitate had formed, while

No. 5 did not commence crystallising until some hours had elapsed; the next day, however, this mixture also contained a rather abundant precipitate.

After the lapse of 1, 2, 4, 8 and 16 days, samples were taken for analysis of the filtrate in the usual manner. The amount of ammonia nitrogen was determined only in the filtrates after eight days' crystallisation, and gave the following results:

No. 1:	26.716 g.	$(NH_4)_2SO_4$	per	100 g.	water
„ 2:	26.658 „	„	„	„	„
„ 3:	26.647 „	„	„	„	„
„ 4:	26.627 „	„	„	„	„
„ 5:	26.644 „	„	„	„	„
Average:	26.658 g.	$(NH_4)_2SO_4$	per	100 g.	water.

The measurements of hydrogen-ion concentrations were also made only in the filtrates after eight days' crystallisation. The results of the analyses are placed together in Table 47 and graphically represented by Fig. 28.

It appears from Table 47, as well as from the above remarks concerning the crystallisation of the experimental mixtures, that the *velocity of crystallisation is higher as the starting concentration of protein increases*. Thus the table shows that Experiment No. 5 is much behind the other experiments after the lapse of one day, and it has hardly reached them after two days.

As for the state of equilibrium, we have repeatedly mentioned that according to the phase rule it is independent of the starting

TABLE 47 (Series of experiments *g*; February 1915).

The experimental mixtures contained 26.658 g. ammonium sulphate per 100 g. water.

No. of Experiment.	Amounts of egg-hydrate in g. per 100 g. water in the experimental mixtures at commencement of the experiment and in the different filtrates.						Hydrogen-ion concentration of the filtrate (*Fourth* filtration)	
	At commencement of experiment	*First* filtration (1 day)	*Second* filtration (2 days)	*Third* filtration (4 days)	*Fourth* filtration (8 days)	*Fifth* filtration (16 days)	$h \times 10^8$	p_H
	g.	g.	g.	g.	g.	g.		
1	14.140	0.841	0.806	0.777	0.768	0.768	13.49	4.870
2	9.419	0.806	0.779	0.750	0.726	0.735	13.18	4.880
3	7.058	0.804	0.745	0.714	0.692	0.718	13.06	4.884
4	4.703	0.806	0.724	0.677	0.654	0.670	13.06	4.884
5	2.350	1.172	0.826	0.677	0.613	0.597	12.97	4.887

concentration of protein, and consequently is was to be expected that the amount of egg-hydrate of the filtrates in the different experiments would approach the same value. On the whole, this may be said to be the case, as appears in a very perspicuous manner from Fig. 27: and this would be still more conspicuous if the scale of the figure was chosen in such a way that also the starting points of the curves corresponding with Experiments Nos. 1 and 2 could have been placed on the figure. A more thorough consideration of Fig. 28, as well as an examination of Table 47, especially of the sixth and seventh vertical columns of the latter, shows, however, if not a great, yet at any rate a distinct decrease in the contents of egg-hydrate of the filtrate from Experiment No. 1 to Experiment No. 5, consequently with decreasing initial concentration of protein; the question then is, where the reason is to be sought for.

Thus the discrepancies in the experiments here described go in the opposite direction of what was to be expected from the usual interpretation above mentioned, according to which the content of egg-hydrate of the filtrate would be the smaller as the starting concentration of protein increases; this interpretation is, we see, not borne out by the results of our experiments. We likewise consider it a fact that the different amounts of egg-

hydrate in the filtrates are not a consequence of a different concentration of ammonium sulphate, since the latter is, as above mentioned, much the same in all the filtrates and is, moreover, found to be greatest in Experiment No. 1, where the content of egg-hydrate is also the greatest. The reason for the decrease established in the contents of egg-hydrate in the filtrates is in our opinion probably to be sought in a circumstance which we have repeatedly pointed out[1]): that crystallisable egg-albumin, if left to stand, is apt to be transformed into non-crystallisable but completely coagulable albumin. We have several times observed that such a transformation can be produced by bacterial action, and it is possible that a similar, but quantitatively less extensive, transformation takes place if the aqueous solutions of egg-albumin are simply left to stand. It is therefore highly probable that an aqueous solution of egg-albumin purified completely by re-crystallisation will after some time always contain a small quantity of non-crystallisable egg-albumin, and consequently the amounts of egg-hydrate

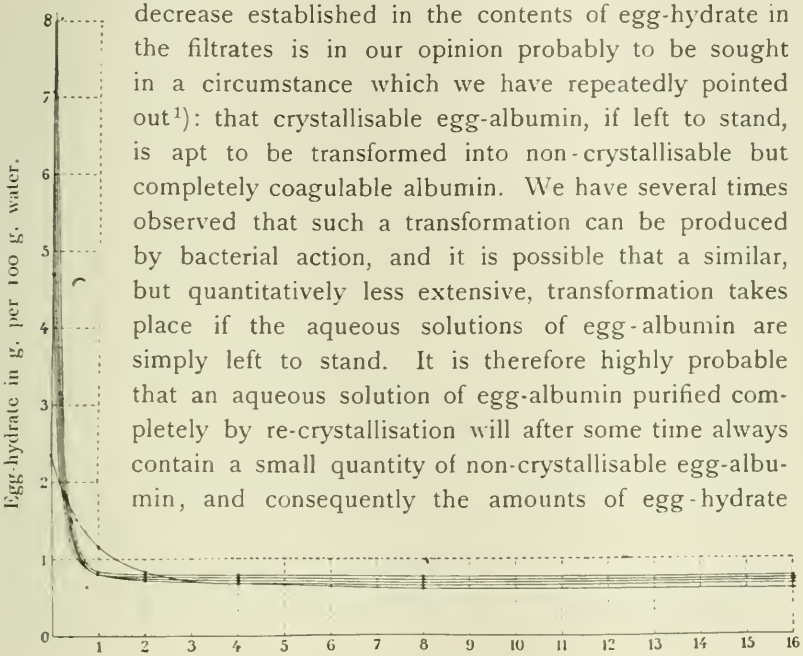

Fig. 28.

which, after crystallisation, are found in the filtrate, will consist partly of the not crystallised part of the crystallisable egg-albumin and partly of the whole of the non-crystallisable part, which latter will of course be the greater the higher the starting concentration of protein. Suppose, for instance, that 1 % of the whole amount of egg-albumin in Series *g* was present in the non-crystallisable state at the moment when the experimental mixtures were prepared: a simple calculation will then show that the contents of crystallisable egg-albumin are nearly the same in all the filtrates after 4 or 8 days' crystallisation (Third and Fourth filtrations):

[1]) Comptes-rendus du Laboratoire de Carlsberg **12**, 22 and 36 (1916).

	Third filtration.	Fourth filtration.
No. 1:	0.777 ÷ 0.141 = 0.636	0.768 ÷ 0.141 = 0.627
„ 2:	0.750 ÷ 0.094 = 0.656	0.726 ÷ 0.094 = 0.632
„ 3:	0.714 ÷ 0.071 = 0.643	0.692 ÷ 0.071 = 0.621
„ 4:	0.677 ÷ 0.047 = 0.630	0.654 ÷ 0.047 = 0.607
„ 5:	0.677 ÷ 0.024 = 0.653	0.613 ÷ 0.024 = 0.589

The reason why we have chosen the Third and Fourth filtrations, and not the Fifth, is that in some of our experiments we found a little more egg-hydrate in the filtrate of the Fifth than of the Fourth Filtration; this is, as mentioned above (see p. 227 and p. 247), a sign of an incipient bacterial action, and consequently supports the explanation given of the question here discussed.

Finally we may ask how far the results of this series of experiments agree with those of the preceding series. A comparison is possible in the case of a single experiment if all the factors influencing the crystallisation are taken into consideration. From Fig. 24, Curves I and II (p. 222), representing the results of Series *a* (Table 39), it appears, that if the crystallisation takes place at indoor temperature, and the starting concentration of protein is about 10.5 g. egg-hydrate per 100 g. water, the filtrate will, if the concentration of ammonium sulphate is 26.658 g. per 100 g. water,

at the end of 4 days contain 0.712 g. egg-hydrate per 100 g. water (Curve I)
and at the end of 13 days contain 0.678 g. egg-hydrate per 100 g. water (Curve II).

In Series *g*, Experiment No. 2, the crystallisation has taken place under practically the same conditions as in Series *a*; only the hydrogen-ion concentration is not the same, since in Series *a* it corresponds with $p_{H^\cdot} = 4.85—4.86$, but in Series *g*, Experiment No. 2, with $p_{H^\cdot} = 4.88$. From Fig. 26 (p. 245) it is now seen that in a filtrate with $p_{H^\cdot} = 4.88$, the amount of egg-hydrate is 1.07 times greater than in a filtrate where $p_{H^\cdot} = 4.85—4.86$.

On the basis of these facts it is thus possible to calculate that the filtrate of Series *g*, Experiment No. 2, must contain

after standing for 4 days: $0.712 \cdot 1.07 = 0.762$ g. egg-hydrate per 100 g. water

and after standing for 13 days: $0.678 \cdot 1.07 = 0.725$ g. egg-hydrate per 100 g. water.

From Table 47 it appears that the *accordance between the content of egg-hydrate really found in experiment No. 2 and the amount calculated on the basis of many earlier experiments is especially satisfactory.*

While in the experiments now described we have used direct crystallisation, just as in Series *a*, we shall in the following series try to throw some light on the question as to the influence exerted by the starting concentration of protein on the state of equilibrium by some experiments in which we adopted the method used in Series *b* — abundant crystallisation and subsequent dissolution of part of the crystallised precipitate.

Series of experiments h. This series, which was carried out in April 1915 at indoor temperature (19°), consisted of four experiments, of which No. 2 and No. 3 were executed in quite the same manner and with mixtures of the same composition. The mixtures were prepared as usual, with a solution of egg-albumin, a "weak" and a "strong" solution of ammonium sulphate, all solutions of known composition.

Samples of the egg-albumin solution were measured and weighed:

for experiment	No. 1:	120 cc.
„ „	„ 2:	60 „
„ „	„ 3:	
„ „	„ 4:	30 „

The amounts of water and "weak" solution of ammonium sulphate required to give Nos. 2, 3 and 4 the same contents of water and ammonium sulphate as No. 1 were then added to the solutions, which were subsequently precipitated by addition of 100 cc. "strong" solution of ammonium sulphate to each mixture. Each solution then contained 27.277 g. ammonium sulphate per 100 g. water, while the amounts of egg-hydrate per 100 g. water varied as follows:

No. 1: 10.433 g.
„ 2: 5.209 „
„ 3: 5.215 „
„ 4: 2.608 „

In all the solutions it was possible, by shaking, completely to dissolve the amorphous precipitates separated by addition of the "strong" solution of ammonium sulphate; yet mixture No. 1 was strongly opalescent. This mixture crystallised well in the course of not more than 1/4 hour, while Nos. 2 and 3 required 1 hour or more, and No. 4 did not crystallise till the next day.

After two days' crystallisation, 60 cc. "weak" solution of ammonium sulphate was added cautiously, while shaking carefully, to each mixture, by which addition the amounts of ammonium sulphate of all the mixtures became 25.115 g. per 100 g. water, while the amounts of egg-hydrate per 100 g. water varied as follows:

No. 1: 8.396 g.
„ 2: 4.192 „
„ 3: 4.198 „
„ 4: 2.099 „

The taking of samples with subsequent analysis was effected as usual, 3 hours, 1 day, 3 days and 8 days after the "weak" solution of ammonium sulphate had been added. Determination of the amount of ammonia nitrogen was only made in the filtrates after the lapse of three days, and gave the following results:

No. 1: 25.146 g. $(NH_4)_2SO_4$ per 100 g. water
„ 2: 25.123 „ „ „ „ „
„ 3: 25.079 „ „ „ „ „
„ 4: 25.113 „ „ „ „ „

Average: 25.115 g. $(NH_4)_2SO_4$ per 100 g. water.

The measurements of hydrogen-ion concentrations were likewise undertaken only in the filtrates at the end of three days. The analytical results are set forth in Table 48.

From Table 48 it appears that the parallel experiments Nos. 2 and 3 coincide completely, the accordance being even better than we should be warranted in expecting from the magnitude of the experimental error. Furthermore the table shows quite a similar

TABLE 48 (Series of experiments *h*; April 1915).

The experimental mixtures contained 25.115 g. ammonium sulphate per 100 g. water.

No. of Experiment.	Amounts of egg-hydrate in g. per 100 g. water in the experimental mixtures after addition of "weak" solution of ammonium sulphate and in the different filtrates					Hydrogen-ion concentration of the filtrate (*Third* filtration)	
	Experimental mixtures	*First* filtration (3 hours)	*Second* filtration (1 day)	*Third* filtration (3 days)	*Fourth* filtration (8 days)		
	g.	g.	g.	g.	g.	$h \times 10^6$	p_H.
1	8.396	1.019	0.987	0.981	0.977	16.44	4.784
2	4.192	0.883	0.882	0.874	0.868	16.75	4,776
3	4.198	0.885	0.879	0.872	0.864	16.14	4.792
4	2.099	0.725	0.760	0.753	0.755	16.33	4.787

picture to that of Table 47; consequently, the remarks relating to the latter are applicable also to Table 48. Also in this case where we are in presence of states of at least approximative equilibrium, the amounts of egg-hydrate of the filtrates increase with increasing starting concentration of protein. The difference in the contents of egg-hydrate of the filtrates is somewhat greater in the latter than in the former series of experiments, but is of course to be accounted for in quite a similar manner to that thoroughly described in the former series.

Accordingly, from the experiments described in this section, we think it justifiable to conclude, that *while the velocity of crystallisation*, as already mentioned above, *increases with the starting concentration of protein, the state of equilibrium is independent of the latter, since the deviations from this rule are only of secondary importance, and can, moreover, be easily and naturally accounted for.*

SUMMARY.

(1) By means of a series of investigations into the crystallisation of egg-albumin by ammonium sulphate we have tried to elucidate the influence exerted by the conditions of crystallisa-

tion both on the *velocity of crystallisation* and on the *state of equilibrium* between the crystallised egg-albumin and the surrounding mother liquor.

(2) The *velocity of crystallisation* increases with the *concentration of ammonium sulphate* (Section A), with the *initial concentration of protein* (Section D) and with the *temperature of crystallisation* (Section B).

(3) In the *state of equilibrium* the content of egg-hydrate of the mother liquor will — under otherwise equal conditions — be the smaller as the *ammonium sulphate concentration* increases (Section A).

The *temperature optimal* to the state of equilibrium of the system (that is, the temperature at which the egg-hydrate concentration of the mother liquor, under otherwise the same conditions, is smallest) is situated between 12^0 and 29^0, probably about 20^0. Yet there is not any essential difference in the state of equilibrium of the system within the limits 12^0 and 29^0, whilst the state of equilibrium at 0^0 is markedly different, since the egg-hydrate concentration of the mother liquor at 0^0 is considerably greater than it is at the higher temperatures. That we have here to deal with *real states of equilibrium*, is proved by the fact that the equilibrium can be displaced to one or another side solely by alteration of the temperature (Section B).

The *hydrogen-ion concentration optimal* to the state of equilibrium of the system (that is, the hydrogen-ion concentration at which the egg-hydrate concentration of the mother liquor, under otherwise the same conditions, is lowest) corresponds with $p_H =$ about 4.58, and seems to be independent of the concentration of ammonium sulphate and the temperature of crystallisation. A decrease of the hydrogen-ion concentration has no influence beyond increasing the content of egg hydrate of the mother liquor, since the appearance and character of the precipitate separated are not altered. An increase of the hydrogen-ion concentration beyond the optimum, on the other hand, results not merely in an increase of the content of egg-hydrate of the mother liquor, but may also give rise to a radical alteration of the appearance and character of the precipitate, if we are in presence of a material increase of the hydrogen-ion concentration (Section C).

The *starting concentration of protein* is without any influence on the state of equilibrium of the system, the discrepancies ob-

served being only of secondary significance, and easily to be accounted for (Section D).

To sum up, the system investigated appears to be in all essential features conformable to the *Phase Rule of Gibbs*; it behaves as a heterogenous system containing one solid phase, and consisting of four components: water, egg-hydrate, ammonia and sulphuric acid.

May 1917.

STUDIES ON PROTEINS.

V. ON THE OSMOTIC PRESSURE OF EGG-ALBUMIN SOLUTIONS.

BY

S. P. L. SØRENSEN.

IN COLLABORATION WITH

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The problem concerning the Osmotic Pressure of colloidal solutions has been investigated by a great number of workers and given rise to numerous publications. If nevertheless it is still far from being set at rest, the reason is mainly to be sought in the extraordinary difficulties encountered by thoroughgoing and accurate research-work in this field, difficulties which relate to the experimental technique required by exact measurements of the osmotic pressure, as well as to the necessity of procuring well defined colloidal solutions. Moreover, an additional difficulty, and none of the least, is due to the circumstance that the composition of such a solution, or, to speak more exactly, the distribution of its constituent parts between the disperse phase and the dispersion medium, is dependent upon a variety of factors, the effects of which often run in opposite directions. Consequently, as the properties of the colloidal solution — and among them its osmotic pressure — are altered with its composition, the whole problem is a most complex and complicated one, and it is therefore not to be wondered at that the results hitherto obtained regarding the osmotic pressure of colloidal solutions present a motley picture from which it is hardly possible to draw trustworthy conclusions regarding the laws governing the phenomena observed.

Thus, for example, when in the case of some colloidal solutions the osmotic pressure is found to increase proportionately to the concentration, whilst in other instances its increase is greater and in still others less than the concentration, one is

tempted to embrace the view expressed by Wo. Ostwald[1]). "Nichts kann vielleicht deutlicher die Unangemessenheit der unbesehenen Übertragung der für molekulardisperse Systeme abgeleiteten "Lösungssetze" auch auf kolloide Systeme demonstrieren, als diese Mannigfaltigkeit der Konzentrationsfunktion des osmotischen Druckes bei Kolloiden". On the other hand, however, it must not be overlooked that, if — as assumed by Ostwald — the phenomenon referred to is chiefly attributable to the degree of dispersity altering with the concentration, and that differently in different solutions, then we have in reality to do with a mere paraphrase of the generally accepted conception of Concentration.

Ostwald (*loc. cit.*, p. 166) admits that the concentration of a colloidal solution cannot be exhaustively defined by simply stating the content per cent of the disperse phase, but that the degree of dispersity must also be stated. But why then — it may be asked — express the relative proportion of the two phases of the colloidal solution by the term "Concentration", which in the chemistry of molecular-disperse substances is used in a very different and quite definite acceptation. The degree of dispersity must be regarded as an essential attribute of the notion of concentration, even though the consequence may be that the concentration of a colloidal solution will be known only in rare cases, so that we shall therefore also be ignorant of the extent to which the physical and chemical constants — including the osmotic pressure — of such a solution alter with the concentration, and of course still more ignorant as to whether the alterations are the same in colloidal as in molecular-disperse and ion-disperse solutions.

The well-known alterations which, for instance, the specific gravity of iodine vapour undergoes with changes of temperature would doubtless never mislead any one to draw the inference that the law of Avogadro does not apply to iodine vapour; on the contrary, it will be found rational from the alterations of specific gravity to draw, on the ground of the said law, conclusions as to the cleavage of iodine molecules with increasing temperature. Neither will the phenomena observed in aqueous solutions of electrolytes be considered as being at variance with

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[1]) Grundriss der Kolloidchemie (Zweite Auflage), p. 296 (1911).

van't Hoff's theory, but, as was first done by Arrhenius, it will be attempted with the application of that theory to clear up the facts connected with the dissociation of electrolytes in aqueous solutions.

We hold that it will be adequate to apply exactly the same view to the investigation of the properties of colloidal solutions. If, for example, the addition of a salt is found to diminish the osmotic pressure of such a solution, it is certainly possible that this is due to a knitting together of the colloidal particles, resulting in a diminution of the degree of dispersity; but it is also conceivable that other factors, such as alterations of hydratation, dissociation *etc.*, have come into play. Hence we consider that the only reliable conclusion which it is justifiable to draw from such an observation is that the addition of a salt tends to diminish the concentration of the substances producing the osmotic pressure, and our task will then be, by a methodical inquiry into the influence exerted by such a factor on the magnitude of the osmotic pressure, to elucidate the nature and extent of the alterations undergone by the colloidal solution, and to find out the cause or causes to which the alterations in the osmotic pressure are due.

As regards the causes which condition what is usually called the "Instability" of a colloidal solution, we are of opinion that they have to be viewed in much the same light. It is a well known fact that the properties of a colloidal solution are liable to alteration by stirring or shaking, as also by heating and subsequent cooling, or simply by the effect of time, and in several other ways: in short, the properties of the solution are dependent on its "previous history". This is, however, nothing but a collective designation fit merely for glossing over our ignorance of the influence exercised by various factors upon the general condition of the colloidal solution, it being all but self-evident that to every alteration observed in the properties of the system corresponds an alteration in the composition or state of the system. Thus for example, the observation made by W. Biltz and A. von Vegesack[1]), that a colloidal solution of benzoepurpurine, poor in electrolytes, exhibits an osmotic pressure which increases if the liquid is stirred, denotes in our opinion

[1]) Zeitschr. physik. Chem., **68**, 367 (1909).

simply that such a system is not in equilibrium. Indeed, the general course of the experiment referred to clearly shows — and the authors themselves are fully aware of the fact — that the system is continually altering during the experiment. The inference drawn by Wo. Ostwald in his comments upon the researches under our notice (*loc. cit.*, p. 295) is, however, not quite legitimate: "Der osmotische Druck der Kolloide ist eine ausserordentlich variable Grösse, die schon entsprechend verschiedener mechanischer Vorbehandlung, dem Alter usw. wesentlich modifiziert werden kann".

To our mind, in the instance considered the only warrantable conclusion is that the colloidal solution was unstable under the conditions of the experiment, whereas the latter has not furnished any information concerning the magnitude of the osmotic pressure exerted by a colloidal solution of benzoepurpurine of well defined composition.

In researches of the kind referred to, the first and very important thing to be done will be to prepare and examine the colloidal solution in such a manner that we are enabled to give an exhaustive definition of its composition, and also, whenever such a solution is to be prepared afresh, to obtain it of identically the same or at any rate of a well-defined composition. One of the most important criteria by which it can be ascertained that we have really obtained a well-defined solution is, we believe, afforded by the circumstance that its physical and chemical constants are the same if the composition of the solution remains constant, and keep unaltered during a period longer than the time required for carrying out the experiments. It seems to us that such a thorough-going critical appreciation of the experimental material is an absolutely necessary, but at the same time sufficient condition of further investigation. It is only after the constant character of the solution has been established — that is, practically speaking, after the limits have been defined within which the physical and chemical properties of the solution are varying if its composition is kept constant —, it is not till then that it will be possible to take up, and carry through successfully, the study of the influence exerted by some definite factor upon any of the properties of the solution. In such a study of colloidal solutions it will in our opinion be advisable to make application, to the greatest extent possible, of the same views

and theories as those applying to real solutions; only in such cases where it appears to be impossible to arrive at an explanation in this way will it be proper to fix our attention on the more or less hypothetical properties which are often mentioned as characteristic of colloidal solutions, and which sometimes only go to show how deficient is our insight into the complex phenomena encountered in colloidal chemistry.

If, for instance, we have to deal with the questions relating to the above-mentioned dependence of osmotic pressure upon concentration, we consider it most suitable to adopt as a basis of our reflections on colloids the theory known from the real solutions, according to which the osmotic pressure is determined by the concentration of non-permeable substances. We are certainly aware that the experimental results will seldom furnish direct and striking evidence of the correctness of such a proceeding: the accordance between experiment and theory will generally be found deficient; yet we prefer to ascribe the causes of the discrepancies to alterations undergone by the concentration of non-permeable substances, rather than attributing to the colloidal substances hypothetical properties that might account for them. The questions then naturally arise, what is the nature and extent of those modifications, to what cause or causes are they due, and in what way are they produced. It is a matter of course that the more simply and clearly these questions can be answered, the more justifiable will our view appear.

Therefore we cannot accede to the view taken by Wo. Ostwald in his "Grundriss der Kolloidchemie" and ultimately resumed as follows (p. 318): "Zusammengefasst besagen diese Erörterungen, dass der osmotische Druck der meisten Kolloide keineswegs eine nur von der Teilchenzahl pro Volumeinheit abhängige Grösse ist, sondern vielmehr stark variiert entsprechend den Zustandsänderungen dieser Systeme, also insbesondere entsprechend den Variationen des Dispersitätsgrades und der Formart der dispersen Phase. Der Wert des osmotischen Druckes ist also bei Kolloiden eine viel komplexere Grösse als bei molekulardispersen Systemen und kann nicht ohne weiteres mit letzterer identifiziert werden. Besonders aber erscheint es aus den genannten Gründen unmöglich, absolute Werte für den osmotischen Druck kolloiddisperser Systeme zu bestimmen.

Zum wenigsten gilt letztere Einschränkung für alle emulsoiden und komplexen Dispersoide, während bei suspensoiden Systemen einfachere Beziehungen, die denen molekulardisperser ähneln, zu gelten scheinen (siehe auch nächsten Abschnitt). Vielleicht wird die zukünftige Forschung es sogar zweckmässiger finden, den Begriff der Osmose für Molekulardispersoide zu reservieren, und für die entsprechenden Erscheinungen bei kolloiden und grob dispersen Systemen einen andern Namen, etwa Hydratation (Solvation) u.s.w., zu wählen, der die wichtigen Unterschiede zwischen beiderlei Phänomenen stets im Auge zu behalten gestattet.«

Ostwald first and foremost aims at elucidating the *difference*, emphasised by him, between colloidal and molecular-disperse systems, whereas to our thinking the best way of furthering the study of colloidal solutions is by basing to the farthest possible extent upon the points of *resemblance* which may be found to exist between these and the real solutions.

With the view of throwing some light on the subject under consideration, we may here give a brief abstract of some of the most important researches hitherto made on the osmotic pressure exerted by those colloidal solutions which are of particular interest to us, *viz.* the protein solutions.

E. H. Starling, in his well-known work on the osmotic pressure of serum proteins[1]), used as "outer liquid" the serum filtrate freed from serum proteins by means of a gelatine filter, and he employed as an osmometer a cylinder of silver-wire gauze bewrapped with several layers of peritoneal membrane and tightened with gelatine. The cylinder was filled with serum filtrate and placed in a glass vessel provided with a manometer and containing the serum solution. The whole was then left to stand in a thermostat at a temperature af about 16°. The osmotic pressure for 1 % protein was in four experiments found to be 3.64 mm of mercury (at the end of 4 days), 4.01 mm (2 days), 2.97 mm (2 days), 4.23 mm (6 days), respectively, while one experiment with a solution of sodium chloride

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[1]) Journ. of Physiol., **24**, 317 (1899); see also *ibid.* **19**, 312 (1896).

(1 %) as outer liquid gave, after 29 hours, an osmotic pressure of 5.29 mm mercury per 1 % protein[1]).

Benjamin Moore and W. H. Parker[2]) principally use parchment membranes, and show that colloidal solutions have a real osmotic pressure, which is not due to crystalloid impurities. They find the maximum osmotic pressure of sheep's serum (water or 0.7—1.0 % sodium chloride solution being used as outer liquid) to vary between 3.9 (water) and 2.5 (salt solution) mm mercury per 1 % protein, the result being thus a little lower than that obtained by Starling. In the case of common egg-white they find a much higher pressure (16.0 to 13.9 mm mercury), while dialysed egg-white gives only a pressure of 9.1 to 7.6 mm mercury per 1 % protein. The experiments were made at the temperatures of 38° and 21°, the osmotic pressure being generally under otherwise equal conditions lower at the former than at the latter temperature.

Referring to a series of experiments on the osmotic pressure of soap solutions, Moore and Parker express the opinion that it is not possible from the osmotic pressure to calculate the size of the molecule, but certainly that of the molecule aggregates existing in the colloidal solutions. These "solution aggregates", as they call them, are formed by a physical association of a greater or smaller number of molecules and constitute a "single osmotic unit". They found that sheep's serum, if heated in a 1 % sodium hydroxide solution, undergoes such an alteration that the osmotic pressure grows five times as great, whence they conclude that a treatment of serum by an alkali results in a very considerable diminution of the "solution aggregates".

E. W. Reid[3]), commenting upon the results arrived at by Starling and Moore & Parker, holds that they do not prove an osmotic pressure to be exerted by the protein contained in serum or egg-white, but simply that these very complex mixtures contain, really dissolved, certain substances, which on ac-

[1]) C. J. Martin (Journ. of Physiol., **20**, 369 (1896)) found by a somewhat similar method that diluted solutions of albumin and hæmoglobin have a slight, but constant osmotic pressure, varying with the temperature in accordance with the gaz law, $P_t = P_0 (1 + \alpha t)$. No numerical data are, however, given in his brief account.

[2]) Amer. Journ. of Physiol., **7**, 261 (1902).

[3]) Journ. of Physiol., **31**, 438 (1904).

TABLE 49.

Reid's experiments on the Influence of Ash content on Osmotic Pressure.

Substance experimented upon.	Ash per cent.	Pressure per 1 % concen. of Proteid in mm of Hg.
Twice crystallised ovalbumin (once washed).....	0.120	3.38
Ditto (many times washed)	0.267	0.00
Salted out egg-albumin (once washed)	0.312	4.82
Ditto (once washed)	0.220	15.71
Salted out ox serum-albumin (many times washed).....	0.633	0.00
Ditto (once washed)	0.461	4.29

count of their impermeability to the membranes used give rise to a measurable osmotic pressure.

Reid himself has made very extensive experiments on various proteins, particularly the albumin of serum and eggs, both purified by crystallisation or "salting out" and subsequent washing; further, serum-globulin, casein and edestin. He used an osmometer similar to that of Starling.

Reid's experimental results offer a motley picture, from which it seems precarious to draw reliable conclusions in any direction.

As an example of the very irregular variations to which Reid finds the osmotic pressure to be subject, we here reproduce (Table 49) one of his tables, which has for its object to show that the pressure is quite independent of the amount of mineral constituents of the preparation experimented upon.

The figures set forth in this table need no further explanation; we may simply point out that the preparation containing the highest amount of ash does not exhibit any osmotic pressure at all.

In accordance with this fact, Reid, summing up the results of his own experiments, states that the osmotic pressure per unit of protein concentration in serum or egg-white is subject

to considerable variation, and this remark applies not only to the non-purified matters, but even to the albumins purified by crystallisation or salting out. The products obtained by washing the albumin salted out or crystallised gave him solutions which did not show any osmotic pressure, and he therefore holds that any osmotic pressure exerted by an albumin solution is due to the presence of certain non-protein substances of otherwise unknown composition, which can be removed by washing with a suitable salt solution.

The case is quite different in a series of researches on the osmotic pressure of hæmoglobin published by Reid in a subsequent paper[1]). In these he uses a membrane of vegetable parchment, and the material experimented upon was dog's hæmoglobin carefully purified by crystallisation and washing. At 15^0 he observed, per 1 % hæmoglobin, an osmotic pressure ranging in seven experiments only between 3.51 and 4.35 mm mercury, from which result he concludes that hæmoglobin is really dissolved in water.

The most thorough-going investigation hitherto made into the osmotic pressure of hæmoglobin is due to G. Hüfner and E. Gansser[2]). As membranes they used the so-called "Diffusionshülsen" as prepared by the firm Schleicher & Schüll, which were previously tested with the greatest care, and the material experimented upon was horse and ox hæmoglobin carefully purified by many re-crystallisations. — In Table 50 the results obtained are reproduced as grouped together by the authors. c signifies the quantity of hæmoglobin in g. contained in one liter of solution; t the temperature; p the osmotic pressure as read direct on the osmometer and expressed in mm of mercury, while p' is the corrected pressure which would have been obtained if it had been possible to prevent the diffusion of water into the hæmoglobin solution in the course of the experiment. Finally, M means the molecular weight of hæmoglobin, calculated by the formula:

$$M = \frac{22.41\ (1 + 0.00366\ t) \cdot 760 \cdot c}{p'}.$$

It appears from Table 50 that the mutual concordance between the experiments is, if not perfect, at any rate sufficient to

[1]) Journ. of Physiol., **33**, 12 (1905).

[2]) Engelmann's Archiv für Physiol., 1907, p. 209.

TABLE 50.

G. Hüfner and E. Gansser's experiments on the Osmotic Pressure of Hæmoglobin solutions.

c.	*t.*	*p* (in mm *Hg*, values directly read).	*p'* (in mm *Hg*, corrected).	*M.*	Nature of the Hæmoglobin.
52.72	10°	62.7	62.97	14780	Horse-hæmoglobin
,,	,,	58.5	58.75	15840	,,
,,	,,	61.1	61.36	15210	,,
,,	,,	63.5	63.77	14630	,,
108.0	1°	109.0	109.9	16790	Ox-hæmoglobin
109.2	,,	114.9	115.9	16110	,,
,,	,,	114.6	115.6	16150	,,
,,	,,	115.5	116.5	16020	,,
,,	,,	116.7	117.7	15860	,,
111.8	,,	122.3	123.3	15500	,,
,,	,,	115.8	116.8	16360	,,
216.0	,,	198.0	201.0	18370	,,
,,	,,	224.0	227.8	16210	,,
,,	,,	218.5	222.2	16620	,,
,,	,,	233.6	237.6	15540	,,

warrant the application of the results to calculations of the magnitude of the molecules.

The average values found for the molecule of horse hæmoglobin (15115) and of ox hæmoglobin (16321) agree satisfactorily with the figures — about 16700 in the case of ox hæmoglobin — obtained by chemical methods (determination of the quantity of hæmoglobin containing one atom of iron or combining with one molecule of carbonic oxide). — Finally, it deserves notice that, as can be easily calculated from the first and fourth columns of the Table, the osmotic pressure found by Hüfner & Gansser per 1% of hæmoglobin is much superior to that measured by Reid (see above, p. 270).

The theory set forth by Benjamin Moore and W. H. Parker (see p. 268) as to the dependence of the osmotic pressure on the colloidal molecule aggregates ("solution aggregates") and the alterations undergone by the osmotic pressure consequent

upon alterations in the crystalloid content of the solution are further explained in a long paper published by Benjamin Moore and H. E. Roaf[1]). They arrived at the conclusion that the osmotic pressure cannot be due to any crystalloid or colloidal substance which is able to diffuse through the membrane. The fact is that they observed first a rapid and then a slow increase of osmotic pressure till the maximum pressure was reached, which will then keep constant for several days, whereas, if due to a slowly diffusing substance, it would diminish after having reached the maximum. Hence, the constant osmotic pressure cannot be attributable to any crystalloids not combined with the colloids in the solution, and as regards such crystalloids as have been attached to the colloids by adsorption or chemical combination and are thus prevented from diffusing through the membrane, they must be considered as "integral constituents" of the colloid[2]). In the absence of such crystalloids, the colloid molecules or aggregates are not stable in the solution; they will unite so as to form complex aggregates large enough to render the osmotic pressure immeasurably minimal. "The function of the crystalloid is to keep the colloid in solution, and by its union with it prevent the formation of too large solution aggregates to give pressure readings, and the function of the colloid to render the crystalloid attached to it indiffusible so that it cannot pass the membrane, and hence gives rise to a pressure" (*loc. cit.*, p. 42). In so far the osmotic pressure may be said to be an effect of the crystalloid matters contained in the colloid aggregates, and

[1]) Biochem. Journ., **2**, 34 (1906).

[2]) A somewhat similar view of the nature of colloidal solutions was advanced by J. Duclaux (Compt. rend. (Paris), **140**, 1468, 1544 (1905); Journ. Chim. physique, **5**, 40 (1907), **7**, 405 (1909). We have only seen a review of these two papers in the Kolloid-Zeitschr., **3**, 126 (1908)). He considers a colloidal solution as consisting of particles or "micelles" surrounded by the intramicellar liquid. The micelles, however, comprehend not only the colloidal substance itself, but also the ions and molecules attached to it. Duclaux, who worked mainly on inorganic colloids, and more particularly on colloidal solutions of ferrihydroxide, holds that the micelles themselves doubtless exert an osmotic pressure of easily measurable magnitude; but the laws governing the pressure are as yet unknown. One of his series of experiments with ferrihydroxide shows that the osmotic pressure increases more than the concentration:

Conc.	1.08	2.04	3.05	5.35	8.86
Press (cm) . . .	0.8	2.8	5.6	12.5	22.6

(Kolloid-Zeitschr., **3**, 134 (1908)).

it will therefore be easily understood that — as was observed by Moore and Parker — an alteration in the salt amount of a solution may give rise to an alteration in the state of aggregation and, consequently, in the osmotic pressure.

The authors, discussing the results obtained by Reid, consider that in those cases where he could not observe any osmotic pressure the protein had by the repeated precipitations and washings been entirely deprived of the alkali and neutral salts contained in the genuine protein, so that its aggregation state had become gradually altered in such a way that at last it did not exert a measurable osmotic pressure.

We may here mention more closely one of Moore and Roaf's experimental results (relating to membranes of vegetable parchment). It concerns the alterations in the osmotic pressure of gelatine solutions as dependent upon alterations of temperature, and shows that the said pressure increases at rising temperature more than what would correspond with an increase proportionate to the absolute temperature, which indicates a dissociation and consequent diminution of the size of the "solution aggregates" as the temperature rises. A prolonged heating to 80^0 or more produces a permanent increase of the osmotic pressure, whereas a shorter heating, whilst giving a higher osmotic pressure for a few days, will afterwards exhibit a decrease down to the primitive level, just as the physical properties of the gelatine solution do not undergo any lasting alterations in consequence of a short heating.

Using as a clue Moore's view, as outlined above, of the interrelations of crystalloids and colloids in colloidal solutions, his laboratory (The Johnston Laboratory, University of Liverpool) carried on during the following years a wide-ranging research-work on the various aspects of the question, and among them the influence exerted by acids and alkalies on the osmotic pressure of serum proteins[1]) and of casein[2]).

It was observed that an addition of slight doses of acid diminished the osmotic pressure of the serum, which was nearly annihilated when the dose of acid added was equivalent to the

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[1]) L. Adamson and H. E. Roaf: Biochem. Journ. **3**, 422 (1908); see also H. E. Roaf: Quart. Journ. Exper. Physiol. **3**, 75 and 171 (1910).

[2]) Benjamin Moore, H. E. Roaf and A. Webster: Biochem. Journ. **6**, 110 (1911).

amount of alkaline matter in the serum ash; on addition of larger quantities of acid the osmotic pressure was found to increase again. An addition of alkali produced an increase of the osmotic pressure of the serum until the alkalinity reached the value 0.37 n; a further addition of alkali resulted in a decrease of the pressure, because under the influence of such high amounts of alkali the serum proteins began to be decomposed while giving off diffusible products.

It was further shown that the relationship of osmotic pressure to the totality of alkali present (both that pre-existing in the serum ash and the dose added) could be expressed by a logarithmic curve.

As for the casein, the special object of the researches was the distribution of the hydroxide added between the colloidal casein solution in the osmometer and the casein-free outer liquid. It was shown that the concentration of free sodium hydroxide as found by titration with phenolphtalein as indicator was higher in the former than in the latter solution, unless the dose of sodium hydroxide was quite minimal, in which case it was entirely fixed by the casein. The maximum of osmotic pressure (129 mm mercury per 1 % of casein) was reached in an experiment where the concentration of free sodium hydroxide in the casein solution was 0.047 n and in the outer liquid 0.025 n.

A summary of the research-work done by the Liverpool school is given by Moore and Roaf in the "Kolloid-Zeitschrift"[1]).

Similar investigations were carried out on similar lines by the American inquirer R. S. Lillie[2]), who, with the use of collodion membranes, specially studied the influence exerted by acids, alkalies and salts, as also by certain non-electrolytes, on the osmotic pressure of gelatine and egg-albumin solutions. Whilst an addition of non-electrolytes does not influence the osmotic pressure, he finds that all electrolytes do alter it, but in widely different ways. By small doses of hydrochloric acid the osmotic pressure of either of the two substances examined is diminished, whereas larger quantities of the same acid are apt to augment the pressure, at all events until a certain limit, beyond which it will go down again. An admixture of potassium hy-

[1]) Kolloid-Zeitschr., **13**, 133 (1913).
[2]) Americ. Journ. of Physiol., **20**, 127 (1907).

droxide tends to increase the osmotic pressure of a gelatine solution, but to diminish that of egg-albumin solutions. Finally, as regards the adjunction of salts, Lillie arrives at the result that it always produces a diminution of the osmotic pressure, and that the diminution is a function of both the ions of the salt. In order to render more apparent the alterations in osmotic pressure observed by Lillie, we reproduce in Tables 51 and 52 two of his series of experiments on egg-white (prepared simply by dilution of natural egg-white with water and filtering off the globulin precipitated).

TABLE 51.

R. S. Lillie's experiments on the influence of Acids and Alkalis on the Osmotic Pressure of Egg-white solutions (*loc. cit.*, p. 141).

Series I: 1.5 % egg-albumin.

No. of experiment.	Electrolyte.	Osmotic Pressure mm mercury.	No. of experiment.	Electrolyte.	Osmotic Pressure mm mercury.
1	0 (Control)	25.6	7	$^{m}/_{3100}$ KOH	24.1
2	$^{m}/_{3100}$ HCl	20.7	8	$^{m}/_{1240}$ KOH	22.6
3	$^{m}/_{1240}$ HCl	11.5	9	$^{m}/_{620}$ KOH	20.2
4	$^{m}/_{620}$ HCl	14.1	10	$^{m}/_{412}$ KOH	18.0
5	$^{m}/_{412}$ HCl	20.4	11	$^{m}/_{310}$ KOH	17.9
6	$^{m}/_{310}$ HCl	22.2			

It will be seen that the results of Lillie's experiments agree in general with those arrived at by Moore and his co-operators; yet he does not endorse the view taken by the Liverpool school concerning the interrelations existing between crystalloids and colloids in a colloidal solution. The view taken by Lillie may be best resumed in his own words: "That there is no essential difference between the conditions of solution of crystalloids and colloids" (*loc. cit.*, p. 132).

Finally, it is to be mentioned that Wolfgang Pauli[1]) and

[1] Wo. Pauli: Die kolloiden Zustandsänderungen der Eiweisskörper (Separat-Abdr. aus Fortschritte der naturwissenschaftlichen Forschung **4**, p. 245 (1912)); Wo. Pauli: Kolloidchemie der Muskelkontraktion (Dresden und Leipzig

TABEL 52.

R. S. Lillie's experiments on the influence of Salts on the Osmotic Pressure of egg-white solutions (*loc. cit.*, p. 144).

Series II: 1.25 % Egg-albumin + Potassium or Sodium salts.

No. of experiment.	Salt.	Osmotic Pressure mm mercury.	No. of experiment.	Salt.	Osmotic Pressure mm mercury.
1	0 (Control)	21.6	8	$^{m}/_{24}$ KCl	4.4
2	$^{m}/_{24}$ NaCl	5.0	9	$^{m}/_{24}$ KBr	4.8
3	$^{m}/_{24}$ NaBr	4.6	10	$^{m}/_{24}$ KJ	5.3
4	$^{m}/_{24}$ NaJ	4.9	11	$^{m}/_{24}$ KNO_3	5.5
5	$^{m}/_{24}$ $NaNO_3$	4.8	12	$^{m}/_{24}$ KCNS	5.7
6	$^{m}/_{24}$ NaCNS	5.3	13	$^{m}/_{24}$ K_2SO_4	3.9
7	$^{m}/_{24}$ Na_2SO_4	4.0			

his co-operators have also, of late years, included the measurement of osmotic pressure in the very extensive researches carried on by them with unflagging energy and published under the common title: "Untersuchungen über physikalische Zustandsänderungen der Kolloide". As an osmometer they use a collodion cap; but they have not given any explicit account either of their working method — which was elaborated by Pauli and M. Samec — or of the results obtained. It appears, however, by the papers quoted above that the authors have measured the osmotic pressure of starch and several of its decomposition products, as also of protein solutions. In the case of the latter, they have studied the dependence of osmotic pressure on the salt-formation and the electrolytical dissociation consequent upon the latter (treatment of protein solutions with acids, bases and salts). By grouping together the alterations found in osmotic pressure with certain simultaneous alterations of physical properties — for instance, the viscosity of the solution —, Pauli and his co-operators have furnished very valuable contributions to our insight into the physico-chemical properties of protein solutions. —

1912), p. 15; Wo. Pauli und Osk. Falek: Biochem. Zeitschr. **47**, 296 (1912); Wo. Pauli: Kolloid-Zeitschr. **12**, 225 (1913); M. Samec und F. von Hoefft: Kolloidchem. Beihefte **5**, 195 (1913); M. Samec und S. Jencic: *ibid.* **7**, 143 (1915).

Although this brief account of the most important researches hitherto published concerning the osmotic pressure of protein solutions clearly shows how precarious is the foundation upon which our knowledge of the phenomena discussed is based, yet it must be considered as an established fact that protein solutions exert a measurable osmotic pressure, the magnitude of which is dependent not only on the nature and concentration of the particular protein, but also on the composition of the dispersion medium, more especially on the electrolytes contained in the solution. The unfortunate circumstance that the results obtained do not justify any farther-going conclusions is principally due to the fact that by far the greatest number of the measurements were effected on materials which had not been previously purified with sufficient care, if at all. In fact, excepting the very careful researches on hæmoglobin solutions and some few of the most recent measurements, the solutions experimented upon are generally of unknown composition, so that the results arrived at by different inquirers are not comparable with one another, while the repetition of a measurement under the same conditions is also rendered out of the question. In the following measurements of the osmotic pressure of egg-albumin solutions we have therefore — as was emphasised above — made a point of working on well-defined solutions, and, moreover, we have considered it as our primary, and not the least important, task to prove that albumin solutions of identical composition always — within the limits of experimental errors — exhibit the same osmotic pressure.

In the foregoing account of the principal researches on the osmotic pressure of protein solutions we have only just touched on the question as to the influence exerted by the crystalloids existing in the solution. This very important problem has been made the subject of a great deal of discussion, which we are not to enter upon here. We need only refer to Moore and Roaf's theory (see p. 272), according to which crystalloids act as stabilising substances in colloidal solutions. In this view of the matter the crystalloids present will exert a considerable influence on the degree of dispersity of the disperse phase and, hence, on the osmotic pressure of the solution. This pressure

may, however, also be affected in another way by the crystalloids — more particularly as far as acids and bases are concerned — *viz.* by the formation of salts which then undergo electrolytic dissociation. This is even the aspect of the matter to which particular importance is attached in recent years by the Liverpool school, and still more so by Pauli and his co-operators. The amphoteric character of protein substances, and the faculty which they consequently possess of forming salts with acids as well as bases — salts which can be electrolytically dissociated like other salts — form the foundation of Pauli's view as to the influence of electrolytes on the osmotic pressure of protein solutions. He considers that the magnitude of the pressure depends not only on the protein concentration, but also on the extent to which salts are formed and on the degree to which these are dissociated. An addition of hydrochloric acid to a protein solution will thus give rise to the formation of electrolytically dissociated protein chlorides, and not solely the colloidal protein ions, but also the chlorine ion fixed by the electrostatic forces in the osmometer will exercise osmotic action.

The first by whom this important question was made the object of thorough-going investigations was F. G. Donnan[1]), who considers the very simple case that a solution of a salt NaR susceptible of being dissociated into Na^+ and R^- is separated from a solution of sodium chloride by a membrane impermeable to the ion R^- and also to the non-dissociated salt NaR, but permeable to all the other ions or salts present. Supposing a complete dissociation to have taken place, we may represent such a system as follows (the membrane being denoted by a vertical line):

Na^+	Na^+
R^-	Cl^-
(1)	(2)

This system is not in a state of equilibrium; but sodium chloride will be diffusing from (2) into (1) until the equilibrium is established, which may by schematised thus:

Na^+	Na^+
R^-	
Cl^-	Cl^-
(1)	(2)

[1]) Zeitschr. für Elektroch. **17**, 572 (1911).

It is now shown by Donnan, through a simple thermodynamic consideration, that, when the equilibrium has been attained, the following equation must be satisfied:

$$[Na^+]_2\ [Cl^-]_2 = [Na^+]_1\ [Cl^-]_1, \qquad (1)$$

where the designations in brackets express molar concentrations of the ions.

In the above considerations Donnan pre-supposes that he is concerned with dilute solutions, and that van't Hoff's law holds good for these. Under the supposition that the substances present are not completely dissociated, and providing that van't Hoff's law also applies to the non-dissociated salt, a similar thermodynamic consideration leads Donnan to the result that the following equation must be satisfied in the state of equilibrium:

$$[NaCl]_1 = [NaCl]_2. \qquad (2)$$

Equation (2) combined with (1) will lead to the expression

$$[Na^+]_1\ [Cl^-]_1 = k\ [NaCl]_1,$$

where k is a constant for any concentration of sodium chloride. As this does not agree with the result arrived at if Arrhenius's method for measuring the degree of dissociation is used, van't Hoff's law is assumed by Donnan — quite gratuitously, we think — to be inapplicable to the non-dissociated salt in the cases under consideration, so that the existence of a state of equilibrium on both sides of the membrane with regard to non-dissociated salt does not necessarily give

$$[NaCl]_1 = [NaCl]_2.$$

In the further course of his considerations Donnan presupposes the correctness of Equation (1), as also a complete dissociation of NaR as well as NaCl, finally the presence of equal volumes of liquid on both sides of the membrane. If now the molar concentration of the compound NaR is designated by c_1, the concentration af sodium chloride in the initial state by c_2, and the concentration of the sodium chloride diffused in the state of equilibrium from (2) to (1) by x, then the concentration of the systems may be summarised as follows: —

Initial state.

Na^+ R^-	Na^+ Cl^-
c_1 c_1	c_2 c_2
(1)	(2)

Equilibrium.

Na^+	R^-	Cl^-	Na^+	Cl^-
$c_1 + x$	c_1	x	$c_2 - x$	$c_2 - x$
(1)			(2).	

By application of Equation (1) to the concentrations in the equilibrium of the system we get:

$$(c_1 + x)\, x = (c_2 - x)^2,$$

whence

$$x = \frac{c_2^2}{c_1 + 2\, c_2}.$$

The *ratio of distribution of the salt*, that is, the relation between the concentration of salt in the solutions (2) and (1) will then be:

$$\frac{c_2 - x}{x} = \frac{c_1 + c_2}{c_2} = 1 + \frac{c_1}{c_2}. \qquad (3)$$

Thus, the *ratio of distribution* will be the greater, the greater c_1 is in proportion to c_2, and it will approach to 1 when c_2 is very great in proportion to c_1. The consequence is, that if there is on one side of the membrane a non-diffusible, electrolytically dissociated substance in sufficiently high concentration, the diffusion of an otherwise readily diffusible substance present on the other side of the membrane will be very susceptible of being checked, so that the concentration of the diffusible substance in the state of equilibrium will be much higher on one side of the membrane than on the other.

The unequal distribution of the salt between the solutions on either side of the membrane must of course be taken into account in the measurement of the *osmotic pressure* exerted by the non-diffusible substance in the state of equilibrium. Donnan deals with this question as follows: —

The true osmotic pressure of the non-diffusible, completely dissociated substance may be expressed thus:

$$P_0 = 2\, c_1 \cdot R \cdot T. \qquad (4)$$

The counter-pressure, P, exerted on account of the unequal distribution of the salt may be expressed:

$$P = 2\, ((c_2 - x) - x)\, R \cdot T. \qquad (5)$$

Hence, the osmotic pressure observed will be:

$$P_1 = P_0 \div P = 2c_1 \cdot R \cdot T \div 2(c_2 \div 2x) R \cdot T = 2R \cdot T(c_1 \div c_2 + 2x). \quad (6)$$

The ratio of the observed pressure to that produced by the non-diffusible substance will consequently be:

$$\frac{P_1}{P_0} = \frac{c_1 \div c_2 + 2x}{c_1} = \frac{c_1 + c_2}{c_1 + 2c_2}, \quad (7)$$

since

$$x = \frac{c_2^2}{c_1 + 2c_2}.$$

The less c_2 is in proportion to c_1, the more will the quotient $\frac{P_1}{P_0}$ approach to 1, and the more, accordingly, will the osmotic pressure measured, P_1, correspond with the total osmotic pressure, P_0, of the two ions of the non-diffusible substance, the ion ($R^{\div}$) and the ion diffusible in itself (Na^+). On the contrary, if c_2 is very great as compared with c_1, the above quotient will approach to the value $^1/_2$, that is to say, the osmotic pressure measured, P_1, will correspond only to one half of the total osmotic pressure, P_0, exerted by the non-diffusible substance, *viz.* that half which is produced by the non-diffusible ion ($R^{\div}$).

The other chapters of Donnan's very interesting paper are of minor importance for the comprehension of the experiments described in the following and may therefore be left unmentioned here.

On the contrary, it deserves notice that Donnan and A. Buckley Harris[1]), by measuring the osmotic pressure exerted by solutions of Congo red, arrived at results which may be accounted for by Donnan's theory. Their researches also throw some light upon earlier experiments due to W. M. Bayliss[2]) and by W. Biltz and A. von Vegesack[3]). More recent experiments made by F. G. Donnan and G. M. Green[4]) as also by F. G. Donnan and Arth. J. Allmand[5]), with semi-permeable membranes of cupric ferrocyanide, and including investigations into the distribution of potassic chloride between two

[1]) Journ. Chem. Soc., **99**, 1554 (1911).
[2]) Proc. Roy. Soc., **81** B, 269 (1909).
[3]) Zeitschr. physik. Chem., **68**, 357 (1909); **73**, 481 (1910); see also W. Biltz: *ibid.* **77**, 91 (1911), and **83**, 625 (1913).
[4]) Proc. Roy. Soc., **90** A, 450 (1914).
[5]) Journ. Chem. Soc., **105**, 1941 (1914).

solutions — separated by such a membrane — of this chloride, to which in one of them was added potassic ferrocyanide, go to show that the state of things, though generally agreeing with Donnan's theory, was rather more complicated than supposed in the same. Nevertheless there can hardly be any doubt but that the foundation upon which it rests is sound and correct, and in all measurements of the pressure exerted by non-diffusible substances in the presence of electrolytes it will therefore be necessary to take into consideration the facts established by Donnan.

His very simple formulæ, as given above, are however not applicable to the undermentioned measurements of the osmotic pressure produced by egg-albumin solutions containing ammonium sulphate. In fact, the state of affairs is here far more complicated than was supposed by Donnan in developing his theory. While considering those complications, we shall therefore now try to apply Donnan's views to egg-albumin solutions containing a salt, for instances sodium chloride, which we choose for the sake of simplicity.

It is first to be observed that egg-albumin is of an amphoteric character and accordingly able to combine both with acids and bases, as mentioned in detail in a previous paper[1]). Consequently, in a salt-containing albumin solution the albumin, within a very wide range of hydrogen-ion concentration, will combine both with the acid and the base corresponding to the salt present. Generally, however, the acid and the base will not be bound up in equivalent quantities; neither can we take it for granted that the albumin salts are completely dissociated, and so it is with the sodium chloride present, provided that its concentration is fairly high. Finally, it may be considered that only a part of the albumin will enter as a constituent of the new salts, so that the total albumin concentration cannot possibly be equal to either of the two albumin salt concentrations, nor to their sum.

If the albumin is designated by the formula RHOH, the simplest salts formed by sodium hydroxide and hydrochloric acid will get the formulæ ROHNa and RHCl. When electrolytically dissociated, these salts give the ions $ROH^{\div}$ and Na^{+}

[1]) Comptes-rendus du Lab. de Carlsberg, **12**, 68 (1917).

and the ions RH^+ and $Cl\div$, respectively. It is possible that still more compounds of egg-albumin and sodium chloride may be formed, such as for example RNaCl, which by electrolytical dissociation might give the ions RNa^+, $RCl\div$, $R\overset{+}{\div}$, Na^+ and $Cl\div$. However that may be, that is, waiving the question as to their real existence or their co-existence in albumin solutions containing sodium chloride, the problem with which we are concerned here will be exclusively this: Is the number of sodium ions dissociated off greater than that of the chlorine ions, or conversely? or perhaps, are they equal in number? In fact, all the other ions contain the albumin rest; accordingly, they are not diffusible, so that they play exactly the same part for the question under consideration as does pure albumin, RHOH. Consequently, the formation of these ions will not give rise to any alteration in the magnitude of the osmotic pressure; neither can they exert any direct influence on the distribution of sodium chloride between inner and outer liquid, this distribution being dependent solely on the quantities of sodium ions and chlorine ions present, according to the above equation of Donnan designated (1):

$$[Na^+]_2\,[Cl\div]_2 = [Na^+]_1\,[Cl\div]_1.$$

Let us now imagine that, in accordance with the above remarks, we have an initial system where the solution (1) contains egg-albumin, RHOH, and the salts formed with the same, in an aggregate concentration which we designate as e, while the concentration of the sodium hydroxide combined with the albumin is called c_1, and that of the hydrochloric acid fixed c_1'. Solution (2) contains only sodium chloride, in concentration designated c_2 as above. In solution (1) both sodium and chlorine ions dissociate off, part of which combine to non-dissociated sodium chloride; at the same time sodium chloride (of concentration x) will diffuse from solution (2) into solution (1). Let $c_1 a$ be the total concentration of sodium ions, in the state of equilibrium, which have been dissociated from the albumin compounds, while the corresponding concentration of chlorine ions is designated $c_1'a'$. If then we suppose $c_1 a > c_1' a'$, the upshot may be expressed by saying that the concentration of sodium chloride dissociated off (present partly in a dissociated and partly in the non-dissociated condition) is $y = c_1' a'$ and that of sodium

ions $= c_1\alpha \div c_1'\alpha'$. Adopting similar designations to those used above, and supposing the volumes of solutions (1) and (2) to be equal, the degree of dissociation of sodium chloride being the same, β, in either solution, then the concentrations in the state of equilibrium may be expressed as follows:

Na^+	$Cl^\div$	Na^+	$Cl^\div$
$c_1\alpha \div c_1'\alpha' + (x + y)\beta$	$(x + y)\beta$	$(c_2 \div x)\beta$	$(c_2 \div x)\beta$

Total concentration of compounds and ions, containing the non-diffusible albumin radical: *e*.

NaCl	NaCl
$(x + y)(1 \div \beta)$	$(c_2 \div x)(1 \div \beta)$
(1)	(2)

Thus, the equilibrium concentrations will be the same as those which we should arrive at if the initial proportion of sodium chloride in solution (1) were less by y, and that of sodium chloride in solution (2) correspondingly greater.

If we now apply Donnan's Equation (1) (p. 279) to the equilibrium system, we shall have:

$$(c_2 \div x)^2\beta^2 = [c_1\alpha \div c_1'\alpha' + (x + y)\beta](x + y)\beta.$$

By substituting in this equation $(c_2 + y) \div (x + y)$ for $c_2 \div x$, and making the calculation, we get:

$$x + y = \frac{(c_2 + y)^2\beta}{2(c_2 + y)\beta + c_1\alpha \div c_1'\alpha'} = \frac{(c_2 + c_1'\alpha')^2}{2(c_2 + c_1'\alpha') + \frac{c_1\alpha \div c_1'\alpha'}{\beta}}.$$

This equation enables us to calculate the *ratio of distribution* of the *salt*; it will be found to be:

$$\frac{c_2 \div x}{x + y} = \frac{(c_2 + y) \div (x + y)}{x + y};$$

or, by substituting the value of $x + y$:

$$1 + \frac{c_1\alpha \div c_1'\alpha'}{(c_2 + y)\beta} = 1 + \frac{c_1\alpha \div c_1'\alpha'}{(c_2 + c_1'\alpha')\beta}. \qquad \text{(a)}$$

As regards the *osmotic pressures*, we arrive at the following results:

P_0, by which we understand the *real osmotic pressure* of the non-diffusible compounds and ions present together with the permeable ions electrostatically fixed, may be expressed thus:

$$P_0 = RT[e + c_1\alpha \div c_1'\alpha']. \qquad \text{(b)}$$

The *counter-pressure*, P, exerted on account of the unequal distribution of the salt, will be:

$$P = RT[(c_2 \div x)(1 \div \beta) \div (x + y)(1 \div \beta) + 2(c_2 \div x)\beta \div 2(x + y)\beta]$$
$$= RT[(c_2 + y) \div 2(x + y)](1 + \beta).$$

Substituting the value of $x + y$, we get:

$$P = RT(1 + \beta)\frac{c_1 a \div c_1' a'}{2\beta + \frac{c_1 a \div c_1' a'}{c_2 + c_1' a'}}. \qquad (c)$$

The osmotic pressure observed, P_1, will then be:

$$P_1 = P_0 \div P = RT\left[e + c_1 a \div c_1' a' \div \frac{(c_1 a \div c_1' a')(1 + \beta)}{2\beta + \frac{c_1 a \div c_1' a'}{c_2 + c_1' a'}}\right], \qquad (d)$$

while

$$\frac{P_1}{P_0} = 1 \div \frac{(c_1 a \div c_1' a')(1 + \beta)}{(e + c_1 a \div c_1' a')(2\beta + \frac{c_1 a \div c_1' a'}{c_2 + c_1' a'})}. \qquad (e)$$

We shall now try to apply the expressions obtained to such egg-albumin solutions as those to be dealt with in the following, the various questions considered being of great consequence for the interpretation of our experimental results in a twofold way. In the first place, it is evident that a counter-pressure originating in an unequal distribution of ammonium sulphate must be taken into account in the study of, for instance, the influence exerted by ammonium sulphate on the magnitude of the osmotic pressure of the egg-albumin; and, secondly, the question as to the distribution of the ammonium sulphate is of paramount importance for the calculation of the factors x, y and z. In a previous paper[1]) we have pointed out that by analyses of the "inner" and "outer liquid" with the aid of the "Method of Proportionality" it is possible to determine the two factors by which the weight of the protein nitrogen has to be multiplied to give the weight of the hydrous egg-albumin containing no ammonium sulphate and the ammonium sulphate fixed by the albumin, respectively.

It is to be remarked, however, that for the Method of Proportionality to be applicable to the case now before us, it is necessary that the outer liquid should be of the same composition as the dispersion medium of the inner liquid, that is, the ammonium sulphate solution in which the particles of albumin

[1] Comptes-rendus du Lab. de Carlsberg, **12**, 47 (1916).

hydrate are suspended. It is therefore of some interest to ascertain to what extent this condition is satisfied.

In the foregoing theoretical considerations we have for the sake of perspicuity assumed the salt present to be sodium chloride; but they are also applicable to ammonium sulphate, which in this respect without doubt behaves in a similar manner to the less complex sodium chloride; there will probably only be some difference in the value of β.

Let us first consider the *ratio of distribution* of the salt, for which we arrived at the following expression ((a), p. 284):

$$1 + \frac{c_1 a \div c_1' a'}{(c_2 + c_1' a')\beta}.$$

This expression — as well as the simpler one set forth by Donnan ((3), p. 280): $1 + c_1/c_2$ — goes to show that, providing c_2 is sufficiently great in proportion to c_1 and c_1', the ratio of distribution will approach to the value 1. From the more complex expression, however, it further appears that the said ratio may even in the case of small values of c_2 approach to 1, *viz.* when the difference $c_1 a \div c_1' a'$ approaches to the value 0. In order to the closer explanation of this result, we will attempt to determine, as far as possible, the distribution of the ammonium sulphate under the conditions of our experiments.

Let us suppose, by way of example, the case that we have before us a system, in the state of equilibrium, whose outer liquid contains an amount of ammonium sulphate corresponding to $S = 7$, that is, 7 g. of ammonium sulphate per 100 g. water, and whose inner liquid contains 1 gram-equivalent (14.01 g.) of protein nitrogen per liter, while showing a hydrogen-ion concentration of about $16 \cdot 10^{\div 6}$. As $S = 7$ corresponds approximately to an ammonium sulphate concentration of $^n/_1$[1]), it follows that c_2 — the initial concentration of ammonium sulphate in the outer liquid — will be nearly equal to 2, since the outer and inner liquids are assumed to be of equal volumes, and since the salt in the state of equilibrium is distributed over double the volume, so that the equilibrium concentration will be only one half of the initial concentration. In a following chapter of this paper (see p. 365, Fig. 37) it will be shown that, when the outer liquid contains 7 g. of ammonium sulphate per 100 g. water, the factor γ, by

[1]) Comptes-rendus du Lab. de Carlsberg, **12**, 158 (Table 32) (1917).

which the weight of the protein nitrogen is to be multiplied to give the weight of the ammonium sulphate[1]) fixed by the egg-albumin, will be about 0.047 when the hydrogen-ion concentration of the inner liquid is about $16 \cdot 10^{\div 6}$. In these conditions the egg-albumin contained in one liter of inner liquid has thus fixed $0.047 \cdot 14.01$ g. of ammonium sulphate, so that, the equivalent weight of ammonium sulphate being 66.07, we get:

$$c_1 = c_1' = 0.047 \cdot 14.01 / 66.07 = \text{abt. } 0.01,$$

provided that the albumin has not, in addition to ammonium sulphate, also bound up surplus sulphuric acid or ammonia. Now we have shown in a previous paper[2]) that at the hydrogen-ion concentration in question (abt. $16 \cdot 10^{\div 6}$) sulphuric acid and ammonia are bound in equivalent quantities, so that we have here $c_1 = c_1' = \text{abt. } 0.01$. The magnitude of β is not known with particular exactness, but may in a practically normal solution of ammonium sulphate be supposed to be very nearly equal to 0.33[3]). As for α and α', we know nothing about their magnitude; but they are doubtless considerably less than 1, and α, the degree of dissociation of the ammonium salt of the albumin, is probably greater than α', the dissociation degree of albumin sulphate.

If we now revert to the expression of the ratio of distribution of the salt:

$$1 + \frac{c_1\alpha \div c_1'\alpha'}{(c_2 + c_1'\alpha')\beta},$$

it will be seen immediately, that with the above values of c_1, c_1', c_2 and β the said ratio can only then be equal to 1, when

[1]) Comptes-rendus du Lab. de Carlsberg, **12**, 41 and 45 (1916).

[2]) *Ibid.*, **12**. 128 (see also Fig. 10) (1917).

[3]) *Ibid.* **12**, 80 (Table 7) (1917). As we are here dealing with an ammonium sulphate solution of so high a concentration that not even the first ammonium group can be supposed to have been completely dissociated, it follows that in the formula (*loc. cit.*, p. 79):

$$\beta = \frac{\beta_1}{2}(1 + \beta_2)$$

$\beta_2 = 0$, and consequently $\beta_1 = 2\beta$. In other words, the ammonium sulphate might here be considered as the ammonium salt of the monobasic acid, HNH_4SO_4, that is, c_2 might be made half and β twice as great. In the present case, where the expression forming part of the formula is $c_2 \cdot \beta$, the result will be the same; but wherever we are dealing with the concentration of a non-dissociated salt and, consequently, with the expression $c_2(1 \div \beta)$, the fact pointed out here is of some consequence (see for example the foot-note p. 293).

the fraction becomes 0, that is, when $a' = a$, and that the maximum deviation from 1 is reached if a' is put equal to 0 and $a = 1$, in which latter case we have the fraction $\frac{0.01}{2 \cdot 0.33}$ $= 0.015$, the ratio thus becoming $= 1.015$ instead of 1. As this limit case will never occur, it may safely be concluded that the error made by putting the ratio of distribution equal to 1 does not exceed 1 % of the real value.

The next question is that relating to the influence exerted by an alteration in the hydrogen-ion concentration when the other conditions are the same as in the instance just described. At a hydrogen-ion concentration higher than about $16 \cdot 10^{\div 6}$ the egg-albumin will bind a somewhat smaller quantity of ammonium sulphate; but at the same time it will bind some surplus sulphuric acid. Thus, if the hydrogen-ion concentration is $25 \cdot 10^{\div 6}$, we shall have $y' =$ abt. 0.036, and c_1 — calculated as above — $= 0.036 \cdot 14.01 / 66.07 =$ abt. 0.008. At this hydrogen-ion concentration, however, the amount of surplus sulphuric acid bound per milligram-equivalent of protein-nitrogen s about 6.5 cc. of $^n/_{1000}$ sulphuric acid (*loc. cit.*, Fig. 10); consequently, in one liter of inner liquid containing 1 gram-equivalent of protein nitrogen, there will be bound, in all, 6.5 cc. of $^n/_1$ surplus sulphuric acid, corresponding with the concentration 0.0065 $^n/_1$. Thus, while $c_1 = 0.008$, we get $c_1' = 0.008 + 0.0065 = 0.0145$. Now, as $a > a'$, it is possible that $c_1 a \div c_1' a'$ may in this case be equal to zero or very nearly zero; if, for instance, $a = 0.66$ and $a' = 0.33$, the said quantity will thus be very near zero. Albeit the values used for a and a' cannot pretend to be called even only approximately correct, yet there can be no doubt but that they are of the right order of magnitude, and that the difference $c_1 a \div c_1' a'$ will accordingly be almost nil; in other words, the ratio of distribution of the salt at the hydrogen-ion concentration $25 \cdot 10^{\div 6}$ will be approximately $= 1$.

At still higher hydrogen-ion concentrations the amount of ammonium sulphate bound up is found to decrease further, while at the same time the surplus sulphuric acid fixed will increase, so that $c_1' a'$ will turn out to be greater than $c_1 a$, and the unequal distribution of the salt will be determined by the magnitude of the difference $c_1' a' \div c_1 a$. The higher the hydrogen-ion concentration, the less the quantity $c_1 a$, and the greater $c_1' a'$,; at a sufficiently

high concentration the term $c_1 a$ may even be discarded. At a hydrogen-ion concentration of, say, $40 \cdot 10^{\div 6}$ the concentration of the surplus sulphuric acid fixed will be found by the above calculation to be c_1' = about $0.014 \cdot {}^{n}/_{1}$, and, if $a' = 0.33$, the ratio of distribution of the salt will be:

$$1 + \frac{0.014 \cdot 0.33}{2.005 \cdot 0.33} = \text{about } 1.007.$$

Thus, even at such a high hydrogen-ion concentration as $40 \cdot 10^{\div 6}$, the error made by putting the ratio of distribution equal to 1 does not exceed 1 % of the real value.

At hydrogen-ion concentrations lower than about $16 \cdot 10^{\div 6}$ the case is somewhat different, as both ammonium sulphate and surplus ammonia will be bound; y' is here the factor by which the weight of protein nitrogen has to be multiplied to produce the weight of the quantity of ammonium sulphate corresponding with the ammonium sulphate fixed as well as with the surplus ammonia fixed[1]). Now, if we imagine the same conditions as above, only with this difference that the hydrogen-ion concentration is but $10 \cdot 10^{\div 6}$, we shall find y' = about 0.053 and, consequently, $c_1 = \frac{0.053 \cdot 14.01}{66.07}$ = about $0.011 \cdot {}^{n}/_{1}$. This concentration, however, corresponds with the whole quantity of ammonia bound, and, as only part of the latter is bound in the form of ammonium sulphate, it follows that the concentration of sulphuric acid, c_1', will be less. Fig. 10 (*loc. cit.*) shows that at a hydrogen-ion concentration of $10 \cdot 10^{\div 6}$ the amount of ${}^{n}/_{1000}$ surplus ammonia bound is about 6 cc. per milligram-equivalent of protein nitrogen, corresponding with a concentration of $0.006 \cdot {}^{n}/_{1}$ (calculated as above). Thus, while $c_1 = 0.011$, we get $c_1' = 0.011 \div 0.006 = 0.005$. In the difference $c_1 a \div c_1' a'$ the latter term will therefore be without material significance since $c_1' < c_1$ and $a' < a$, and at still lower hydrogen-ion concentrations we need only reckon with the quantity $c_1 a$. The alteration in the ratio of distribution of the salt is here of comparatively greater significance than at high hydrogen-ion concentrations, because a is much greater than a'. If the above values are used, the ratio of distribution at the hydrogen-ion concentration $10 \cdot 10^{\div 6}$ will be:

[1] Comptes-rendus du Lab. de Carlsberg, **12**, 45 (1916).

$$1 + \frac{0.011 \cdot 0.66 \div 0.005 \cdot 0.33}{2.002 \cdot 0.33} = 1 + \frac{0.017}{2.002} = \text{about } 1.009.$$

Thus, if putting the ratio of distribution equal to 1, we make an error which already at a hydrogen-ion concentration of $10 \cdot 10^{\div 6}$ nearly reaches 1 % of the real value and is still more considerable at lower concentrations.

In the foregoing examples we have throughout been reckoning with an albumin concentration corresponding with one gram-equivalent of protein nitrogen per liter of inner liquid — — a rather strong solution (about 9 g. anhydrous egg-albumin in 100 cc. solution) — and with an outer liquid containing 7 g. of ammonium sulphate per 100 g. water. We now proceed to inquire into the question as to the extent to which the ratio of distribution of the salt is influenced by an alteration in the concentration of protein or ammonium sulphate.

In regard to the albumin concentration, it is evident that the quantities of ammonia and sulphuric acid bound: c_1 and c_1', respectively, are in a direct proportion to this concentration, and the error — defined as above — then appears from the expression found for the distribution ratio of the salt to be only $1/n$ of the value stated above, if, under otherwise equal conditions, the albumin concentration is made n times lower.

When the concentration of ammonium sulphate is higher than the value corresponding with $S = 7$, the error under otherwise the same conditions will likewise be *less* than stated before. In fact, c_2 will be greater, whilst — as seen from Fig. 37 (p. 365) — y and consequently c_1 and c_1' do not increase further with increasing S. β will in this case of course decrease a little; but so will α and α' too, so that the only important consequence of S becoming greater will be an increase in the value of c_2, and accordingly a diminution of the fraction in the expression of the distribution ratio.

At considerably lower concentrations of ammonium sulphate than that corresponding with $S = 7$ the error turns out, on the contrary, a little greater than indicated above. Indeed, it appears by Fig. 37 (p. 365) that y, and consequently c_1 and c_1' decrease with the ammonium sulphate concentration, but in a less measure than the latter, so that the fraction $(c_1\alpha \div c_1'\alpha') / (c_2 + c_1'\alpha')\beta$ will increase as the values of c_2 decrease. When $S = 0.7$ — corresponding with a value of c_2 10 times as low as in the above example —, y will be about 0.013 (for $h = 16 \cdot 10^{\div 6}$), while

in that example y was 0.047; hence, c_1 and c_1' are but 3 to 4 times less than above, whereas c_2 is 10 times less, and the error will consequently be about 3 times as great. At still lower ammonium sulphate concentrations the error will be still greater, but our researches at so low concentrations are not numerous enough to authorise sure quantitative conclusions.

The principal results of our considerations may be summed up as follows: —

The ratio of distribution of ammonium sulphate between the outer and the inner liquid is, under the experimental conditions chosen, generally very nearly equal to 1, and especially so, the lower the albumin and the higher the ammonium sulphate concentration. At a hydrogen-ion concentration a little higher than the isoelectric point of egg-albumin, the difference $c_1 a \div c_1' a'$ *is equal to zero and, consequently, the ratio of distribution exactly equal to 1, whether the concentration of protein and ammonium sulphate be high or low. At any other hydrogen-ion concentration the ratio is greater than 1, the deviation from this value being greater at a diminution than at an increase of the said concentration.*

As indicated above (p. 285), the calculation of the factor r by means of the Method of Proportionality[1]) gives perfectly correct results only when the distribution ratio of the salt is exactly equal to 1, while a higher value of the said ratio will give a correspondingly lower value of the ratio a_b / a_f. Hence, the factor r — which in the case of an outer liquid destitute of protein is calculated from the formula:

$$r = 100 \ (1 \div a_b / a_f) / p_b$$

— will be too great; but an *error of 1 % in the value of* a_b / a_f will, as explained in the paper quoted above (*loc. cit.*, p. 43) produce in *the value of r an error considerably greater than 1 %*.

The next question is, how does the "*Donnan counter-pressure*" produced by an unequal distribution of salt make itself felt in the measurement of the osmotic pressure exerted by egg-albumin solutions containing ammonium sulphate?

The osmotic pressure observed, P_1, may be expressed as follows by means of our formula (d) (p. 285):

$$P_1 = RT \left[e + c_1 a \div c_1' a' \div \frac{(c_1 a \div c_1' a')\ (1 + \beta)}{2\beta + \frac{c_1 a \div c_1' a'}{c_2 + c_1' a'}} \right].$$

[1] Comptes-rendus du Lab. de Carlsberg, **12**, 41 (1916).

Now, if $(c_1 a \div c_1' a')$ is nil, in which case the ratio of distribution of the salt is exactly equal to 1 (see p. 286), the expression found for P_1 is simplified to:

$$P_1 = R \cdot T \cdot e,$$

that is to say, the cause of the osmotic pressure observed is to be sought exclusively in the non-diffusible ions and non-dissociated compounds present; in other words, it is proportionate to the concentration of egg-albumin. The difference $(c_1 a \div c_1' a')$ has been shown above to be reduced to zero at a hydrogen-ion concentration slightly higher than the isoelectric reaction of the albumin and estimated at $25 \cdot 10^{\div 6}$ or there about. In all measurements of osmotic pressure effected at this hydrogen-ion concentration the pressure observed may therefore be supposed to be proportionate to the albumin concentration. If now the osmotic pressure per unit of egg-albumin is found to be altered in consequence of some factor or other being altered, we shall be justified in drawing the conclusion that this latter alteration has caused an alteration in the albumin concentration, or expressed in another way, an alteration in the number of the albumin particles. If, for instance, the osmotic pressure per unit of egg-albumin decreases with increasing concentration of ammonium sulphate, it may be inferred that a condensation of albumin particles takes place as the ammonium sulphate concentration increases.

The case is more complicated when the osmotic pressure is measured in a solution whose hydrogen-ion concentration is considerably higher or lower than $25 \cdot 10^{\div 6}$, in which case the quantity $(c_1 a \div c_1' a')$ is not nil. According to our expression for P_1, this quantity will be greater or less than $R \cdot T \cdot e$, according as $(c_1 a \div c_1' a')$ is greater or less than the fraction

$$\frac{(c_1 a \div c_1' a')\ (1 + \beta)}{2\beta + \dfrac{c_1 a \div c_1' a'}{c_2 + c_1' a'}}$$

and P_1 will be equal to $R \cdot T \cdot e$ even if $(c_1 a \div c_1' a')$ is not nil, provided that

$$c_1 a \div c_1' a' = \frac{(c_1 a \div c_1' a')\ (1 + \beta)}{2\beta + \dfrac{c_1 a \div c_1' a'}{c_2 + c_1' a'}}.$$

After abbreviating the common factor $(c_1 a \div c_1' a')$, a simple calculation will give the equation the following form:

$$c_1 a \div c_1' a' = (1 \div \beta)\ (c_2 + c_1' a') = (1 \div \beta)\ (c_2 + y).$$

That is to say, *even if* $(c_1 a \div c_1' a')$ *is not nil,* P_1 *will nevertheless acquire the value* $R \cdot T \cdot e$, *provided that the concentration of the non-diffusible ion electrostatically fixed in the inner liquid is equal to the sum of the concentrations of non-dissociated salt in the outer and inner liquids*[1]). This result enables us to form a fairly accurate estimate of the extent to which the osmotic pressure observed will be altered with the ammonium sulphate concentration when the hydrogen-ion concentration is different from $25 \cdot 10^{\div 6}$, that is when $(c_1 a \div c_1' a')$ is different from zero.

As long as we have to do with quite minimal values of c_2, it is evident that β will be approximately equal to 1, so that

$$c_1 a \div c_1' a' > (1 \div \beta)(c_2 + c_1' a').$$

The *osmotic pressure observed* will in this case be *greater than* $R \cdot T \cdot e$. *If* c_2 *increases,* β will decrease and, consequently, the value of the product $(1 \div \beta)(c_2 + c_1' a')$ rise very considerably, so as to become equal to $(c_1 a \div c_1' a')$ at a suitable value of c_2, when we shall accordingly have $P_1 = R \cdot T \cdot e$. At *still higher* values of c_2 the said product will become greater than $(c_1 a \div c_1' a')$ and, hence, P_1 *less than* $R \cdot T \cdot e$; but at the same time, as a and a' are continually decreasing with increasing values of c_2, the expression $(c_1 a \div c_1' a')$ will approach to zero, so that P_1 will *approach* to the value $R \cdot T \cdot e$ when c_2 becomes *sufficiently high.*

Further, the extent to which the osmotic pressure is influenced by alterations in the hydrogen-ion concentration can be estimated by the aid of the expression:

$$c_1 a \div c_1' a' \gtreqless (1 \div \beta)(c_2 + c_1' a').$$

Supposing c_2 to be constant, and the hydrogen-ion concentration to be sufficiently low, the value of $c_1 a$ will be so high (and that of $c_1' a'$ so low) that the signs $=$ and $<$ are to be discarded. P_1 will then be greater than $R \cdot T \cdot e$. If the hydrogen-ion concentration increases, $c_1 a$ will be found to decrease and $c_1' a'$ to increase; accordingly, P_1 will diminish, and become equal to $R \cdot T \cdot e$ when $(c_1 a \div c_1' a') = (1 \div \beta)(c_2 + c_1' a')$. At still higher hydrogen-ion concentrations we shall find $(c_1 a \div c_1' a')$ to be less than $(1 \div \beta)(c_2 + c_1' a')$; P_1 will then be less than $R \cdot T \cdot e$, pass

[1]) As the above expression concerns the concentration of non-dissociated salt, the values of c_2 and $(1 \div \beta)$ should be replaced by $c_2/2$ and $(1 \div \beta_1)$, at any rate when the ammonium sulphate concentrations are rather high (see the foot-note p. 287).

through a minimum and increase again till it reaches $R \cdot T \cdot e$ at $(c_1 a \div c_1' a') = 0$, which, as mentioned above, may be presumed to be the case when the hydrogen-ion concentration is about $25 \cdot 10^{\div 6}$.

At still higher hydrogen-ion concentrations we shall have $(c_1 a \div c_1' a') < 0$, and our assumption (see p. 283) that $c_1 a > c_1' a'$ will then hold good no more. It is, however, self-evident that all the foregoing considerations may be repeated in the case that $c_1' a' > c_1 a$, putting $c_1 a = y$. The expression to be entertained in this case will evidently be:

$$c_1' a' \div c_1 a \gtrless (1 \div \beta)(c_2 + c_1 a);$$

and in the same way as above it is obvious that so long as $(c_1' a' \div c_1 a)$ differs but slightly from zero, this difference will be less than $(1 \div \beta)(c_2 + c_1 a)$; but as the hydrogen-ion concentration increases, it will rise until reaching and even exceeding the value of this product. Accordingly P_1 will first be less than $R \cdot T \cdot e$, pass through a minimum and then increase again to the value $R \cdot T \cdot e$; at still higher hydrogen-ion concentrations this increase will continue, yet at a slower rate than at the low hydrogen-ion concentrations, because — as mentioned above (p. 287) — a' is less than a. Hence, the *system of curves expressing the dependence of the osmotic pressure on hydrogen-ion concentration* under otherwise equal conditions will exhibit *two minima, one on either side of a maximum* representing the value $R \cdot T \cdot e$.

In conclusion, we have to inquire into the influence exerted by the albumin concentration on the osmotic pressure per unit of albumin. We consider our expression (d) (p. 285):

$$P_1 = R\,T\left[e + c_1 a \div c_1' a' \div \frac{(c_1 a \div c_1' a')(1 + \beta)}{2\beta + \frac{c_1 a \div c_1' a'}{c_2 + c_1' a'}}\right]$$

and suppose that c_2 is not so high as to completely push back the dissociation of the albumin salt, yet sufficiently high to effect that the dissociation degrees a and a' are determined exclusively by its magnitude, but remain unaffected by the albumin concentration and the concentrations c_1 and c_1'. The inquiry then arises, if under these conditions P_1 will become n times greater if e is made n times greater, while the concentrations of ammonium sulphate and hydrogen-ions are kept constant. An inspection of the terms of the above expression one after the other

will show that e as well as $c_1 a$ and $c_1' a'$ become n times greater, as c_1 and c_1' are proportionate to e, while a and a' are constant. The fraction, however, does not become n times greater, although the numerator gets n times greater; for the denominator increases at the same time, though not in proportion. Now, as the fraction enters into the expression as a negative term, we arrive at the ultimate result, that under the conditions in question, that is, at *not too low concentrations of ammonium sulphate*, P_1 *will increase more than the albumin concentration.*

But if the value of c_2 is sufficiently high, the consequence will be, as stated above, that the difference $(c_1 a \div c_1' a')$ approaches to zero, P_1 thus acquiring gradually the value $R \cdot T \cdot e$. Accordingly, providing the concentrations of ammonium sulphate are sufficiently high, P_1 *will increase in the same proportion as the albumin concentration.*

In the second limit case: that in which c_2 is nil or quite minimal as compared with $c_1' a'$, we likewise find that c_1 and c_1' increase in proportion to the albumin concentration; but a and a' are not constant, but dependent on the magnitude of c_1 and c_1', so as to decrease in an inverse ratio to c_1 and c_1'. Hence it follows that in our expression (d), which in the case now before us may be conveniently written thus:

$$P_1 = R\,T\left[e + (c_1 a \div c_1' a')\left(1 \div \frac{1 + \beta}{2\beta + \frac{c_1 a \div c_1' a'}{c_2 + c_1' a'}}\right)\right],$$

only e will be n times greater when the albumin concentration gets n times greater, whereas $(c_1 a \div c_1' a')$ will increase more slowly. — Finally, as regards the quantity:

$$1 \div \frac{1 + \beta}{2\beta + \frac{c_1 a \div c_1' a'}{c_2 + c_1' a'}},$$

it will remain practically constant at an increasing albumin concentration, since β, if c_2 is quite minimal, may be put equal to 1 and the fraction $(c_1 a \div c_1' a') / (c_2 + c_1' a')$ is but slightly affected by an increasing albumin concentration, provided that c_2 is infinitesimal as compared with $c_1' a'$.

To sum up, the result obtained is, that, supposing *the concentration of ammonium sulphate to be very low*, P_1 *decreases at a slower rate than the albumin concentration.* If this is confronted with the result arrived at for somewhat higher concentrations of

ammonium sulphate, which was the exact reverse, it is directly evident that there must exist an *intermediate suitable concentration of ammonium sulphate at which the osmotic pressure observed increases in proportion to the albumin concentration.*

In the following experimental chapter, where we group together the results of the experiments we have made up to this time, we shall deal in greater detail with the question as to whether those results may be said to tally with the principles set forth by Donnan, which we have made the basis of the above considerations.

Section A will give a complete description of the method used for the measurement and calculation of the osmotic pressure of egg-albumin solutions, and the causes of error incident to it will also be exposed.

Section B has for its subject the question as to whether the osmotic pressure exerted by an egg-albumin solution of a given composition is subject to variation or not.

In *Sections C. D* and *E* we deal with the dependence of the osmotic pressure on the concentrations of ammonium sulphate, hydrogen-ions and protein matter, respectively.

Finally, *Section F* describes our mode of calculating the factors r. x, y and z.

A. The Method used for Measuring the Osmotic Pressure.

The various methods employed in the progress of time for the measurement of osmotic pressure have been described by Ernst Cohen and J. W. Commelin[1]), and more recently by Alexander Findlay[2]). Referring our readers to the historical accounts given by these authors, which, as it were, complete each other, we may limit ourselves to a few remarks illustrative of the differences existing between our procedure and those generally used.

By far the greater part of the measurements hitherto made of the osmotic pressure were realised by the aid of different

[1]) Zeitschr. physik. Chem. **64**, 1 (1908).

[2]) A. Findlay: Der osmotische Druck (German translation by Guido Szivessy). 1914.

apparatus enabling the operator to directly measure the pressure gradually produced in an osmometer provided with a manometer — but otherwise closed — by reason of the dissolvent diffusing through the semi-permeable membrane; the osmotic pressure of the inner liquid will then be represented by the maximum pressure thus reached. This is the way in which were carried out all the measurements of osmotic pressure of protein solutions mentioned in the introduction to the present paper. The same remark applies — as far as can be seen by the accounts published up to the present, which are not very rich in details — to Wo. Pauli's researches on the same subject. In this connection may also be mentioned, as being based upon the self-same principle, the very ingenious apparatuses contrived by H. N. Morse[1]) and his co-operators, whose initiatory researches on the osmotic pressure of the sugars are well known.

In contradistinction to the above-mentioned methods we have elaborated, in co-operation with Mr. J. A. Christiansen, a kind of compensation method, the principle of which consists simply in determining the counter-pressure to be exerted on the inner liquid in the osmometer in order to compensate the osmotic pressure of the solution. Seeing now that, when the counter-pressure is lower than the osmotic pressure of the inner liquid, there will continually be penetrating a current of outer liquid through the membrane into the inner liquid, whilst at a higher counter-pressure the current goes in the opposite direction, it follows that the object of our method must be to determine, at what counter-pressure there does not occur any penetration from either side, because of the counter-pressure being equal to the osmotic pressure of the inner liquid.

This principle was applied for the first time by G. Tammann[2]) in some determinations of the osmotic pressure of cane-sugar. We need not, however, enter into the details of his procedure, which, as was acknowledged by himself, was rather deficient in various respects; suffice it to say that he used as a membrane cupric ferrocyanide precipitated on an unglazed clay cylinder. So did, after him, Berkeley and E. G. J. Hartley[3]), who measured the osmotic pressure of several sugars by the aid

[1]) H. N. Morse: The osmotic pressure of aqueous solutions. Washington (1914).
[2]) Zeitschr. physik. Chem. **9**, 97 (1890).
[3]) Phil. Transact. (London) A. **206**, 481 (1906).

of an improved apparatus constructed on the same principle, and whose research-work, which is still continued[1]), may be in more than one respect put on a level with that accomplished by Morse and his co-operators.

To our knowledge, such low osmotic pressures as those occurring in the protein solutions generally examined have never before been measured by the help of any apparatus based upon the said principle. The following is a complete description of the apparatus employed for our measurements, which are also explained in detail.

a. The Apparatus and Procedure used.

The apparatus is outlined in Fig. 29. The osmometer consists of a collodion cap, *A*, placed on a short glass tube, *B*, into the upper part of which a ground glass tube fits perfectly, so that no water can ooze through; this tube is narrowed a little above the ground part to a capillary tube divided into millimeters, starting from zero on the tube *B*. The capillary tube is provided with a glass cock, *C*, permitting to shut off the space below *C*. A fuller description of the osmometer is given on p. 303, where its use is also explained.

The osmometer, as well as a stirring apparatus, *D*, moved by electricity, and a Beckmann thermometer, *E*, are lodged in an unsilvered Dewar vessel, which is surrounded by a cylinder glass, *F*, immerged in a well regulated water thermostat (not seen in the figure, but described p. 306).

By means of an indiarubber tube, *G*, the osmometer can be hermetically connected with the counter-pressure apparatus, the use of which is shown by the figure. The reservoirs *H* and *I* are partially filled with water and communicating with each other by means of an indiarubber tube. By raising or lowering the former reservoir, it is possible to alter at will the pressure on the water in the latter and, consequently, the counter-pressure in the osmometer. The magnitude of the pressure at any moment can be read on the manometer, *M*, which is filled with water and provided with a rule divided in millimeters (not seen in the figure).

If now we are to measure the osmotic pressure of a protein

[1]) Proc. Roy. Soc. (London) A. **92**, 477 (1916).

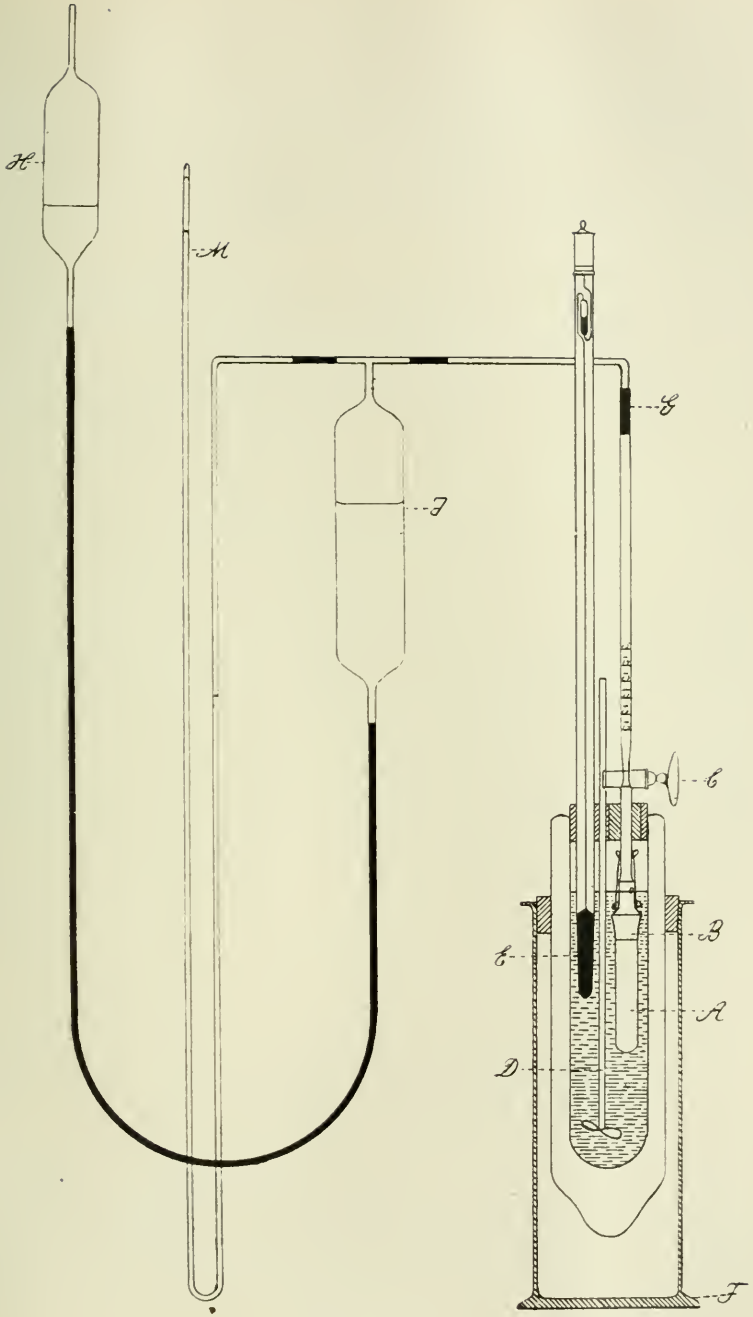


Fig. 29.

solution, the apparatus just described is filled[1]) to a little above the cock C with the solution, which then forms the "inner liquid", while the "outer liquid" is poured into the Dewar vessel in such quantity as just to reach the zero on B. The outer liquid must be as far as possible of the same composition as the dispersion medium contained in the inner liquid, so as to realise a "diffusion equilibrium" between the diffusible components of the two liquids (see p. 307).

If the osmometer is made to communicate with the counter-pressure apparatus (the cock C being open); if, further, by raising or lowering H, a counter-pressure, p_m (measured in centimeters water-pressure), is exerted on the surface of the protein solution in the capillary tube, and if the pressure due to the difference of level between the inner and outer liquids and which can be read by the divisions of the said tube, is equal to p_e (converted into centimeters water-pressure with due allowance for the specific gravity of the protein solution as also for the capillary action; see p. 306), — then the total counter-pressure will be $p_m + p_e$. Supposing the outer liquid to have the right composition, the osmotic pressure of the inner liquid — abstractedly from the "Donnan counter-pressure" which may chance to occur — will equal the total counter-pressure required to completely poise the whole system, so that the surface of the liquid in the capillary tube of the osmometer will neither rise nor sink. Accordingly the measurement, having for its object to determine the total counter-pressure at which the level in the capillary tube will keep constant, may be undertaken at once, if the composition of the outer liquid is absolutely adequate; but this is rather a rare case.

Generally, after filling in the outer liquid up to the zero, it will be preferable, while keeping the cock C closed, to wait until a perfect "diffusion-equilibrium" has been established the next day and not to proceed with the measurement till then.

As regards the measurement itself, it is to be noted that the velocity with which the liquid rises or falls in the capillary tube has proved to be proportionate to the difference between the osmotic pressure and the total counter-pressure, in any case when this difference is not too great. Our immediate object is

[1]) For the proceeding, see p. 304.

therefore simply to measure the velocity with which the level of the liquid in the capillary tube alters in response to a given counter-pressure. For the sake of making the procedure as sensitive and at the same time as little time-wasting as possible, the alterations undergone by the level are read with the aid of a special microscope equipped with a micrometer scale, the division of which is such that 33.8 of its least divisions correspond with one millimeter.

Supposing now that, in the course of a definite length of time — say in the time unit adopted by us, ten minutes —, the level subjected to the counter-pressure p_1 rises v_1 division lines of the micrometer, and that, against the counter-pressure p_2 exerted during the same period, the level rises v_2 lines, then we shall have:

$$\frac{P \div p_1}{v_1} = \frac{P \div p_2}{v_2} = \frac{p_1 \div p_2}{v_2 \div v_1} = a.$$

where P is the osmotic pressure.

a — the value of which is evidently dependent on the transverse section area of the capillary tube — is a quantity characteristic of each particular collodion cap, depending on its permeability and surface area, and varying but little from day to day. It denotes *the magnitude of the over- or under-pressure required to produce*, in the course of *10 minutes*, an *alteration in the capillary level of one micrometer division line*. This magnitude can, as shown by the formula, be determined from two measurements, and P can then be calculated by a single measurement, thus:

$$P \div p_1 = a \cdot v_1$$
$$P = p_1 + a \cdot v_1.$$

Supposing that the level has in t minutes risen u division lines, the rise in 10 minutes will be:

$$v = 10 \cdot u / t,$$

and the above expression thus acquires the general form:

$$P = p + a \cdot 10\, u / t, \qquad \text{(I)}$$

p being the sum of p_m and p_e.

If u is reckoned with a sign, so that a rise in the capillary tube in reckoned as positive, a fall as negative, the Formula (I) may be employed for the calculation of the osmotic pressure on the basis of any measurement of this kind.

If we put together graphically a series of measurements, using the counter-pressures (p_1, p_2, etc.) as abscissæ and the alterations

in the capillary level per 10 minutes (v_1, v_2, etc.) as ordinates, then the points found will — providing that our suppositions are correct, and that there is really a "diffusion-equilibrium" between the inner and outer liquid — form a straight line, whose point of intersection with the axis of abscissæ gives the osmotic pressure P, this counter pressure corresponding with the alteration 0. As will be easily seen, the value of α is also determined as soon as the straight line has been drawn. This graphical method is a little more circumstantial than the use of Formula (I), but affords an excellent survey of the mutual accordance of the measurements.

It is a matter of course that the exactness of the method largely depends on the constancy of the temperature; if the latter is varying in the course of the operations, the osmometer will act as a thermometer. Therefore, as mentioned above, we have taken due care to maintain practically the same temperature by placing the osmometer in a Dewar vessel, lodged in turn in a carefully regulated thermostat. Further, as also mentioned, we have placed in the outer liquid a Beckmann thermometer permitting to read in thousandth parts of degrees the alterations undergone by the temperature in the course of the measuring process, and it has proved possible to regulate the temperature (see p. 306) in such a way that the alteration it undergoes in the outer liquid amounts to only a few thousandths of a degree in several hours.

Moreover, the correction necessitated by the alteration in temperature can be easily calculated if we know the transverse section area of the capillary tube, s, the expansion-coefficient of the liquid, δ, the internal volume, r, of the osmometer: the correction per one thousandth of a degree, k_τ, will then be:

$$k_\tau = r\,\delta \,/\, 1000\, s^{1}).$$

For the four apparatus employed, k_τ expressed in the divisions of the micrometer scale was found to be 0.020, 0.044, 0.078 and 0.085, respectively, and the correction to be applied

1) It is to be remarked, however, that, strictly speaking, this calculation is only approximately correct, inasmuch as allowance has not been made for the circumstance that the temperature of that part of the capillary tube which overreaches the Dewar vessel may not be the same as that shown by the Beckmann thermometer, and may also alter differently. In calculating the correction in question, we have not taken this circumstance into account, since it concerns but an insignificant part of the total volume of the inner liquid.

to each measurement is arrived at by simply multiplying k_τ by the alteration of temperature expressed in thousandths of degrees. Of course, we must here also reckon with the sign, and if the correction relating to an elevation of temperature is reckoned as positive, it must be subtracted from the directly measured alteration in the capillary level. If the latter alteration is designated by h (expressed in the units of the micrometer scale, a rise being reckoned as positive), and the elevation of temperature as τ (reckoned in thousandths of degrees, and a rise reckoned as positive), we get:

$$h - k_\tau \cdot \tau = u.$$

It is this value, u, of the alteration of capillary level, after correction for temperature alteration, which enters into Formula (I) (p. 301).

In executing the last-mentioned measurement, we always took care to keep the inner liquid for some time — generally two or three hours — under the exact counter-pressure corresponding with the osmotic pressure.

Subsequently, we took samples for analysis of the inner and outer liquids. For this purpose, after closing the cock C, and taking the osmometer out of the outer liquid, we removed the upper part of the osmometer and took out very quickly, by means of a pipette of exactly known volume, about 5 cc. of the inner liquid. The sample was weighed while the remaining part of the inner liquid was decanted and used for measurements of hydrogen-ion concentrations. Lastly, some samples of the outer liquid were measured off and weighed, and in the samples of either liquid the contents of ammonia nitrogen and protein nitrogen were estimated by the method described in a previous paper[1]). Finally, the amounts of "coagulable" and "non-coagulable" protein nitrogen were estimated in 100 cc. of the outer liquid.

b. Details concerning Apparatus and Method.

1. The *Osmometer.* Figures 30 and 31 show the principal parts of this apparatus on a larger scale.

The collodion cap, A, of about 15 cc. capacity, is made in exactly the same manner as the larger caps used in the dialysis apparatus[2]), only with this difference that the test tube used is

[1] Comptes-rendus du Lab. de Carlsberg, **12**, 48 (1916).

[2] *Ibid.*, **12**, p. 28 (1916).

smaller. With regard to the collodion it is to be remarked that it has not been possible this year's time to obtain a commercial preparation that, even after previous purification (*loc. cit.*, p. 28) would be suitable for this use. The collodion we used, which has proved excellent, was prepared by Mr. H. Jessen-Hansen by nitration of cotton-wool, according to the recipe of W. Biltz and A. von Vegesack[1]).

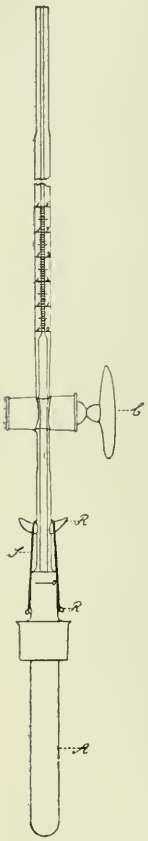


Fig. 30.

The manner in which the cap is fastened is seen in Fig. 31. After cautiously drawing up the cap over the lower part of the short glass tube whose upper part, marked in the figure as *P*, is ground internally, we surround the cap and glass tube with a piece of tight-fitting indiarubber tube *O*, enclosed in turn in a close glass limb *N*. In this manner we have easily succeeded in making the osmometer perfectly tight, at all events against the pressures used. Fig. 31 also shows the ground tube *Q*, which fits exactly into *P* and is continued in the divided capillary tube.

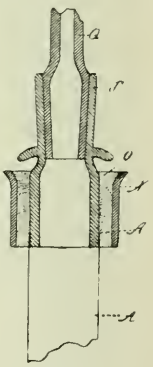


Fig. 31.

In Fig. 30 it is indicated, how the capillary tube is kept in the right position by means of projecting glass pins *R* and indiarubber strings *S*. The zero from which the division of the capillary tube is reckoned is also seen in the figure.

When not in use, the cap, with the glass tube, is kept in toluol water. Prior to each experiment the cap is carefully washed with the solution to be used as "outer liquid", and is then entirely immersed in pure outer liquid for some ten minutes, at the end of which the liquid is decanted as completely as possible. The cap is now filled with inner liquid to the upper edge of the glass tube, which must be done very cautiously by the aid of a pipette, to avoid the formation of air-bubbles. Then the capillary tube is fixed cautiously, the cock *C* being left open and due care taken to obviate any formation of air-bubbles while the

[1]) Zeitschr. physik. Chem., **68**, 364 (1909).

inner liquid is rising in the tube[1]). When the capillary tube has been put in place, the inner liquid is completed up to about the middle of the divided part of the capillary tube, by the aid of a capillary pipette ending in a long taper which can reach through the whole length of the tube and also through the cock[2]).

Then the indiarubber strings *S* are fastened on, and the osmometer placed in the outer liquid in the Dewar vessel (see Fig. 29, p. 299); subsequently, the cock *C* is closed, and the quantity of outer liquid is proportioned so as to raise it very exactly to the zero of the capillary tube.

After the latter has finally been connected through the indiarubber tube *G* (Fig. 29) with the counter-pressure apparatus, the whole system is ready for a measurement to be undertaken. As before mentioned (p. 300), it is, however, recommendable to leave it in repose (the cock *C* remaining closed) for some hours or, preferably, till the next day, in order to bring about a perfect equilibrium of temperature and diffusion.

It is further to be observed that prior to proceeding with the experiment it is necessary to shake the outer liquid with toluol and then to filter it. During the whole course of the experiment a current of air deprived of carbonic acid and saturated with toluol must be conducted through the outer liquid; it must only be discontinued at the moments when the measurements are effected. Especially if the inner liquid has prior to the beginning of the experiment been saturated with toluol, it will be found that the air current saturated with the same substance,

[1]) It goes without saying that the capillary tube must be previously cleaned and dried. It is also necessary to rub vaseline or cock's fat on the ground part of the tube; but any excess must be carefully removed, as it might give rise to the formation of air-bubbles.

[2]) The manipulation here described does not offer any material difficulty, providing the operator has acquired some practice, and observes certain precautions: The capillary pipette should be carefully wiped on the outside before being introduced into the capillary tube; the taper of the pipette must be completely filled with liquid, and the outflow must not begin till the pipette dips in the liquid; by this means the formation of air-bubbles is prevented. If some air-bubbles should nevertheless form in the tube or at the cock, they must be removed with the aid of a platinum wire or a clean and dry capillary pipette, as even small bubbles may become the cause of gross errors. Their presence will soon make itself felt, it being impossible to obtain concordant results of the measurements, even if the latter are made at very short intervals.

which will diffuse through the collodion cap, is sufficient to protect the outer as well as inner liquid from the action of putrefaction bacteria, even if the operations extend over ten or twelve days. An incipient putrefaction will immediately manifest itself by an elevation of osmotic pressure. It is not practical to use toluol in such quantity that the surface of the outer liquid would be covered with a layer of this substance, which might impede an accurate taking of samples of outer liquid at the end of the experiment, it being also to be noted that liquid toluol is very injurious to the indiarubber strings *S*.

2. The *Thermostat* used consists of a rectangular copper case, provided in two opposite sides with a pane (of the same height as the case) enabling us to see through the thermostat. The dimensions of the latter are such (33 · 33 · 42 cm.) that there is room enough for two cylinder glasses (marked *F* in Fig. 29), each containing a Dewar vessel with an osmometer. In all the experiments we have effected hitherto, a temperature of about 18^0 was maintained by slowly letting on water from the conduct and heating by an electric glowing-lamp, which is kindled and extinguished automatically with the help of a common electric temperature regulator. The variation of temperature from day to day was generally found to be much less than $0^0.1$. We need not add that the thermostat was equipped with an agitator.

3. The rise produced by the *capillary action* in the capillary tube of the osmometer is determined by means of another capillary tube of known diameter. For this determination we use the portion of inner liquid left when the osmometer has been filled, disregarding such slight modifications in capillary action as may be entailed by the alteration which the composition of the inner liquid may undergo in the course of the experiment. Supposing that in measuring the capillary action in a capillary tube of a diameter of b mm. the liquid has risen a cm., and supposing further the capillary tube of the osmometer to have a diameter of b' mm. and the specific weight of the inner liquid to be d_e, then the correction relating to the capillary action, expressed in cm. water-pressure, will be

$$a \cdot d_e \cdot b \,/\, b'.$$

c. Description of a Measurement.

The mode of proceeding may be best explained in detail by the description of the measuring and analytical operations carried out in the course of an experiment (marked No. 61).

The liquids to be examined were a solution of egg-albumin which had been re-crystallised six times, and an about 7 n solution of pure ammonium sulphate. The analysis of these two liquids had shown that:

100 g. of egg-albumin solution contained:

2.0678 g. protein nitrogen
2.6585 g. ammonia „ ,

and that:

100 g. ammonium sulphate solution contained:

8.1371 g. ammonia nitrogen.

For the re-calculation of protein nitrogen to egg-albumin hydrate we adopted the factor 8.4, while disregarding the ammonium sulphate fixed by the albumin-hydrate, this proceeding being sufficiently exact for such a preliminary calculation, the chief object of which is to enable us to give the outer liquid a composition identical or at least similar to that of the dispersion medium of the inner liquid. If now for the re-calculation of ammonia nitrogen to ammonium sulphate we employ, as usual, the factor 4.7163, then:

100 g. albumin solution will contain:

17.370 g. egg-hydrate
12.538 g. $Am_2 SO_4$
70.092 g. water;

100 g. ammonium sulphate solution:

38.377 g. $Am_2 SO_4$, 61.623 g. water.

The inner liquid used in the experiment was prepared in a flask of 25 cubic centimeters capacity, and consisted of about 20 cc. albumin solution (weight: 22.203 g.), about 1 cc. ammonium sulphate solution (weight: 1.154 g.) and water up to the mark (weight of the water: 4.065 g.). Thus, the inner liquid had the following composition:

	Egg-albumin hydrate.	$Am_2 SO_4$.	Water.
22.203 g. egg-albumin solution:	3.857 g. +	2.784 g. +	15.562 g.
1.154 g. $Am_2 SO_4$ „ :		0.443 g. +	0.711 g.
4.065 g. Vand			4.065 g.
27.422 g.	3.857 g. +	3.227 g. +	20.338 g.

TABLE 53.

(Experin

Apparatus No. II with Cap No. 6; specific gravity of inner liquid (d_e): 1.093 (see p. 3

The apparatus was put toge

Date; No. of measurement.	Duration of measurement, in minutes. t	Alteration measured of capillary level during the time t. h	Mean temperature read on Beckmann thermometer. $T_{Beckm.}$	Temperature change read during measurement, in $^1/_{1000}$ degrees. τ	(Correction per $^1/_{1000}$ degree, $k_\tau = 0.020$). $k_\tau \cdot \tau$	Alteration, measured and corrected, of capillary level during time t. $h \div k_\tau \cdot \tau = u$	Alteration of capillary level $\frac{10\,u}{t} = v$
Oct. 5; 1	9	÷11.7	2.566	+ 2	+ 0.04	÷11.7	÷13.
,, 2	19	÷13.4	2.568	+ 3	+ 0.06	÷13.5	÷ 7.
,, 3	12	÷ 3.4	2.568	+ 2	+ 0.04	÷ 3.4	÷ 2.
,, 4	21	÷ 0.5	2.570	0	0	÷ 0.5	÷ 0.
,, 5	25	÷ 2.0	2.570	0	0	÷ 2.0	÷ 0.
,, 6	21	÷ 1.9	2.567	÷ 5	÷ 0.10	÷ 1.8	÷ 0.
,, 7	10	+ 1.1	2.561	÷ 3	÷ 0.06	+ 1.2	+ 1.
,, 8	40	+ 3.3	2.555	÷11	÷ 0.22	+ 3.5	+ 0.
Oct. 6: 9	30	÷ 5.1	2.535	÷ 2	÷ 0.04	÷ 5.1	÷ 1.
,, 10	19	÷ 1.2	2.533	÷ 1	÷ 0.02	÷ 1.2	÷ 0.
,, 11	20	+ 0.8	2.532	÷ 2	÷ 0.04	+ 0.8	+ 0.
,, 12	16	+ 0.2	2.530	÷ 1	÷ 0.02	+ 0.2	+ 0.
,, 13	12	+ 1.3	2.529	÷ 1	÷ 0.02	+ 1.3	+ 1.
Oct. 7; 14	26	+ 3.1	2.490	+ 1	+ 0.02	+ 3.1	+ 1.
,, 15	39	+ 2.2	2.493	+ 4	+ 0.08	+ 2.1	+ 0.
,, 16	23	÷ 1.2	2.496	+ 3	+ 0.06	÷ 1.3	÷ 0.
,, 17	21	÷ 3.8	2.499	+ 2	+ 0.04	÷ 3.8	÷ 1.
,, 18	36	+ 0.3	2.501	+ 1	+ 0.02	+ 0.3	+ 0.
Oct. 8; 19	16	+ 1.3	2.501	0	0	+ 1.3	+ 0.
,, 20	11	÷ 0.8	2.502	+ 1	+ 0.02	÷ 0.8	÷ 0.
,, 21	19	÷ 1.0	2.502	0	0	÷ 1.0	÷ 0.
,, 22	26	+ 0.2	2.504	+ 2	+ 0.04	+ 0.2	+ 0.
,, 23	135	÷ 0.1	2.510	+11	+ 0.22	÷ 0.3	0

61).

illary action: 0.78 cm. (see p. 306); temperature (C°): $T_{Beckm.} + 15^{0}.71$.

October 3, 1914, at 4^{45} o'clock.

read on manometer, in cm. p_m	Height of liquid in capillary tube of osmometer, in cm. h_e	Pressure of liquid in capillary tube of osmometer, in cm. water pressure (d_e means the specific gravity of the solution). $h_e \cdot d_e = p_e$	Total counter-pressure, in cm. water-pressure. $p_m + p_e = p$	Magnitude, in cm. water-pressure, of α	Magnitude, in cm. water-pressure, of $\alpha \cdot v$	Osmotic pressure in cm. water-pressure. $p + \alpha \cdot v = P$	
9.50	14.12	15.43	74.93		÷ 7.54	(67.39)	
6.97	14.12	15.43	72.40		÷ 4.12	68.28	
4.59	14.12	15.43	70.02	0.58	÷ 1.62	68.40	
3.02	14.11	15.42	68.44	(found	÷ 0.12	68.32	Mean: 68.13
3.00	14.11	15.42	68.42	graphi-	÷ 0.46	67.96	Capillary
3.00	14.11	15.42	68.42	cally,	÷ 0.52	67.90	action 0.78
1.98	14.11	15.42	67.40	see Fig. 32)	+ 0.70	68.10	67.35
1.98	14.12	15.43	67.41		+ 0.52	67.93	
2.54	14.68 [1])	16.05	68.59	0.64	÷ 1.09	67.50	
1.92	14.68	16.05	67.97	(found	÷ 0.38	67.59	Mean: 67.46
.30	14.69	16.06	67.36	graphi-	+ 0.26	67.62	Capillary
.30	14.69	16.06	67.36	cally,	+ 0.06	67.42	action 0.78
.40	14.69	16.06	66.46	see Fig. 32)	+ 0.70	67.16	66.68
.29	14.80	16.18	66.47	0.60	+ 0.72	67.19	
.73	14.81	16.19	66.92	(found	+ 0.30	67.22	Mean: 67.26
.52	14.81	16.19	67.71	graphi-	÷ 0.36	67.35	Capillary
.09	14.79	16.17	68.26	cally,	÷ 1.08	67.18	action 0.78
.14	14.79	16.17	67.31	see Fig. 32)	+ 0.06	67.37	66.48
.15	14.80	16.18	67.33	0.54	+ 0.43	67.76	
.77	14.80	16.18	67.95	(found	÷ 0.38	67.57	Mean: 67.59
.67	14.80	16.18	67.85	graphi-	÷ 0.27	67.58	Capillary
.31	14.80	16.18	67.49	cally,	+ 0.05	67.54	action 0.78
.31	14.80	16.18	67.49	see Fig. 32)	0	67.49	66.81

[1]) See p. 310.

Hence, the inner liquid contained:

18.964 g. egg-albumin hydrate per 100 g. water
15.867 g. Am_2SO_4 . . . . . „ „

and the outer liquid had now to be prepared so as to have such a composition that the proportion of ammonium sulphate per 100 g. water would be practically the same as in the inner liquid.

The outer liquid was accordingly prepared with

		Am_2SO_4.	Water.
179.70 g. Am_2SO_4 solution,	containing	68.963 g.	+ 110.737 g.
321.50 g. water	„		321.500 „
501.20 g.		68.963 g.	+ 432.237 g.

The outer liquid consequently contained

15.955 g. Am_2SO_4 per 100 g. water.

The inner and outer liquids prepared were shaken with toluol. After they had been left to stand in an ice-safe, the excess of toluol was removed by filtration, and the liquids were then placed again in the ice-safe. Immediately before being poured into the osmometer, they were heated to about 18^0.

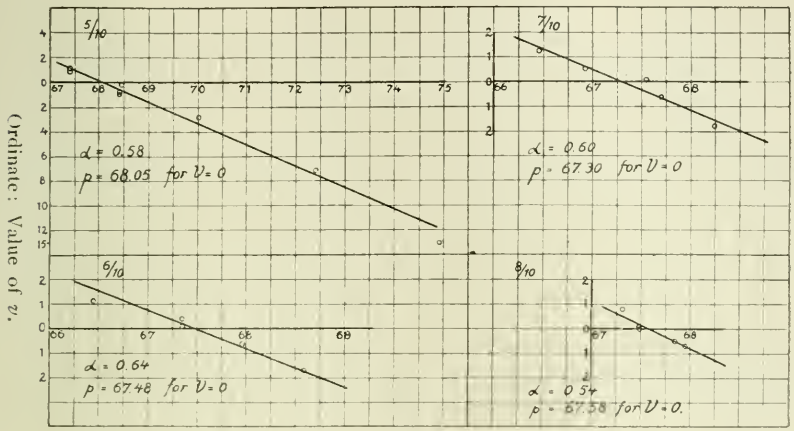
The measurement was effected with the aid of Apparatus No. II with Cap No. 6. The apparatus was filled and joined together on the 3rd of October 1914, when the cock of the capillary tube was closed. The measurements were carried out from the 5th to the 8th October.

The results are tabulated pp. 308 & 309.

The above description of our procedure will suffice to explain both this Table 53 and the accompanying Fig. 32. It is only to be remarked that in the night between the 5th and 6th of October the liquid in the capillary tube had risen about 2 cm., probably because the cock had not been quite close. Before proceeding with the first measurement, on the 6th October, we therefore reduced the quantity of liquid, by the aid of a pipette, approximately to the level of the day before.

From the result of the measurement it appears that, in the course of the two days intervening between the filling of the apparatus and the first measurement, a well-nigh complete diffusion equilibrium has been established, the osmotic pressure keeping practically constant during the whole time of the mea-

suring operations. The slight depression which was observed with certainty to have taken place in the osmotic pressure between the 5th and 6th October can, however, hardly be attributed to the aforementioned very inconsiderable increase of volume, but is probably due to the circumstance that the diffusion equilibrium was not perfectly established till the 6th. —



Abscissa: Value of p. Fig. 32.

After the completion of the measurement, the next step was to take out samples for analysis.

5 cc. of inner liquid was taken out by means of a pipette, No. 1 [1]), and weighed in a measuring flask of 100 cc.; the liquid was then diluted with water up to the mark and weighed again. After shaking carefully, 3 · 20 cc. of the diluted solution were weighed off for analysis:

Weight of the 5 cc.	Weight of the 100 cc.	Weight of the 20 cc.
5.4980 g.	100.212 g.	Flask No. 36: 20.069 g.
		— · 39: 20.065 g.
		— · 44: 20.054 g.
		mean value . . . 20.063 g.

[1]) The weight of 5 cc. water measured at indoor-temperature with Pipette No. 1 being 5.032 g., the specific gravity of the inner liquid (d_e, see Table 53) was calculated, with sufficient exactness for our purpose, at 5.4980 : 5.032 = 1.093.

The thiosulphate solution used in the titration had the concentration $1.0035 \cdot n / 14.01$. The amount of ammonia and protein nitrogen, respectively, was found to correspond with

Ammonia nitrogen.	Protein nitrogen.
Flask No. 36: 27.69 cc.	17.94 cc.
— · 39: 27.64 ·	17.82 ·
— · 44: 27.63 ·	17.94 ·
Mean value . . . 27.653 cc.	17.90 cc.

Thus, *100 g. of inner liquid contained:*

$$\frac{27.653 \cdot 1.0035 \cdot 100.212 \cdot 100}{20.063 \cdot 5.4980 \cdot 1000} = 2.5210\ g.\ \textit{Ammonia nitrogen}\ (a_b)$$

and, calculated in the same way, 1.6319 *g. Protein nitrogen* (p_b).

From the outer liquid 100 cc. were measured off and weighed in a measuring flask of 100 cc., then diluted with water up to the mark and weighed again. After shaking carefully, 3 · 8.5 cc. of the diluted solution were weighed for analysis[1]).

Weight of 10 cc. outer liquid.	Weight of 100 cc.	Weight of 8.5 cc.
10.785 g.	100 563 g.	Flask No. 46: 8.555 g.
		— · 54: 8.544 g.
		— · 83: 8.609 g.
		Mean value . . . 8.569 g.

No protein nitrogen was found. In the titration the amount of ammonia was found to correspond with:

Flask No. 46: 26.65 cc.
— · 54: 26.63 ·
— · 83: 26.86 ·
Mean value . . . 26.713 cc.

100 g. outer liquid thus contained:

2.9169 g. Ammonia nitrogen (a_f).

Finally, 100 cc. of outer liquid was found to contain 0.08 mg.

[1]) The quantity taken out for analysis was so proportioned that the amount of ammonia in each sample was practically the same as in the samples of inner liquid (see Comptes-rendus du Laboratoire de Carlsberg, **12**, 48 (1916)).

"coagulable" and less than 0.1 mg. "non-coagulable" protein nitrogen.

Electrometrically the hydrogen-ion concentration was found to be:

In the inner liquid: $h = 13.58 \cdot 10^{\div 6}$; $p_H = 4.867$
- - outer - : $h = 17.91 \cdot 10^{\div 6}$; $p_H = 4.747$.

Thus, the result of the experiment here described is that *an ammonium-sulphate-containing solution of egg-albumin of the composition and the hydrogen-ion concentration of the inner liquid at 18°.21 (2°.50 + 15°.71) is in perfect equilibrium with an ammonium sulphate solution of the composition and hydrogen-ion concentration of the outer liquid, if the albumin solution is subjected to a pressure equal to that of a 66.81 cm. high water column at indoor temperature (20°). This pressure is therefore a measure of the osmotic pressure exerted by the albumin solution under the actual conditions.*

From the values found for a_b, p_b, a_f and p_f (the value of p_f is in this instance equal to zero) it is of course possible to calculate r by the formula

$$r = \frac{100\,(a_f \div a_b)}{a_f \cdot p_b \div a_b \cdot p_f}\ ^{1}).$$

In the present instance we get

$$r = \frac{100\,(2.9169 \div 2.5210)}{2.9169 \cdot 1.6319} = 8.32.$$

d. Calculation of the Dependence of Osmotic Pressure on Egg-albumin concentration.

The form in which we have above comprised the result of a measurement of osmotic pressure simply represents the values found by direct measurement. In dealing with the results of a considerable number of experiments, however, it is necessary, for the sake of comparison, to re-calculate the values in such a manner as to make the results illustrative of the dependence of the osmotic pressure on the albumin concentration.

The simplest way of expressing albumin concentration is that commonly adopted, in which the concentration, c, is reckoned

[1]) Comptes-rendus du Laboratoire du Carlsberg, **12**, 41 (1916).

as proportionate to the amount of protein nitrogen contained in volume unit of the solution, the osmotic pressure P being reckoned as proportionate to c, so that P will be equal to $R \cdot T \cdot c$. It is thus taken for granted — just as we have done, for simplicity's sake, in our considerations regarding the "Donnan counter-pressure" — that van't Hoff's law holds good of the solutions considered. It is to be remarked, however, that this law applies solely to very weak solutions, where the volume of the dissolved substance is quite minimal as compared with that of the dissolvent; but in the case of stronger solutions it is not certain that the law will hold true, and we have therefore thought necessary to take into account the volume of the egg-hydrate.

As has been explained in the introduction to this series of papers[1]), the just-mentioned reason has induced us to state the albumin concentration, in general, not in proportion to the volume of the solution, but to the weight or volume of the dispersion medium.

On the same occasion it was mentioned that by the ammonium sulphate content of the dispersion medium, S, we understand the weight, in g., of the ammonium sulphate per 100 g. of water in the dispersion medium, — and by the egg-hydrate concentration, E, the number of milligram-equivalents of protein nitrogen per 100 g. water in the dispersion medium. In such cases — as for instance in determinations of the magnitude of osmotic pressure — where it is convenient to reckon with the egg-hydrate concentration, ε, per volume unit of dispersion medium, we shall have ε equal to E/V_s, where V_s means the volume at 18°, in cc., of the weight of dispersion medium containing 100 g. water[2]).

If now we provisionally suppose the magnitude of the osmotic pressure to be proportionate to the concentration ε, as defined above, and if we suppose that each albumin molecule or albumin particle acting as a molecule contains n nitrogen atoms, then we shall have

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[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 8 (1915).

[2]) Concerning the calculation of these quantities on the basis of the analytical results, we refer to the Introduction (*loc. cit.*, p. 8) and Table 32 (Comptes-rendus du Laboratoire de Carlsberg, **12**, 158 (1917)), as also to the calculation given in detail below (p. 318).

$$P = \frac{R \cdot T \cdot \varepsilon}{n} = \pi \cdot \varepsilon$$

and, hence,

$$\pi = \frac{P}{\varepsilon} = \frac{P \cdot V_s}{E},$$

where π $(= \frac{R \cdot T}{n})$ signifies the osmotic pressure per milligram-equivalent protein-nitrogen per cubic centimeter of dispersion medium. The pressure being measured in cm. of water, and the volume unit used being the cubic centimeter, we get, at the temperature 18°:

$$R \cdot T = \frac{p_0 \cdot v_0}{T_0} T_{18} = \frac{22.412 \cdot 76 \cdot 13.596 \cdot 1 \cdot 291}{273} = 24685 \text{ cm.}$$

A. Findlay[1]), in his very careful and explicit account of the modes of calculation of osmotic pressure, sets up the following equation, which he has deduced by the thermodynamic way and which is applicable to ideal solutions:

$$P = \frac{R \cdot T}{V_o}(x + \frac{x^2}{2} + \frac{x^3}{3} + \text{— — -- — }),$$

where V_o means the molecular volume of the dissolvent in cc. at the pressure of one atmosphere, while x denotes the molar fractional part of dissolved matter, or, in other words, the ratio of the number of dissolved molecules to the total number of molecules present.

In the case of the solutions used in the present investigations this equation may be simplified so as to assume the form employed by us above. Let E signify the number of milligram-equivalents protein-nitrogen per 100 g. water in the dispersion medium, and let n denote the number of nitrogen atoms in an albumin molecule. The number of milligram-molecules of egg-albumin in the volume of solution which contains 100 g. water will then be E/n, and, if we designate by a and s the number of milligram-molecules of water and ammonium sulphate, respectively, in this volume[2]), then the molar fraction, x, of dissolved matter can be expressed thus:

$$x = \frac{\frac{E}{n}}{\frac{E}{n} + a + s}.$$

Now, as E/n is always quite minimal and at any rate infinitesimal as compared with $a + s$, it follows that x can be put equal to $\frac{E}{n}/(a + s)$. Further, as x under these conditions gets only a minute

[1] A. Findlay: Der osmotische Druck; deutsche Ausgabe von G. Szívessy, 1914, p. 39.

[2] a will of course always be 100 000 / 18.02 = about 5550, whereas the magnitude of s depends on the amount of ammonium sulphate, S.

value, it is evident that in Findlay's equation all the terms containing x in a higher power than 1 may be discarded without any appreciable error slipping in. The equation thus assumes the following form:

$$P = \frac{R \cdot T}{V_o} \cdot \frac{E}{n\,(a + s)}.$$

As we reckon with milligram-molecules, V_o signifies the volume of one milligram-molecule of the dissolvent, or, in the present case, of the dispersion medium, which consists of an ammonium sulphate solution containing in the volume V_s a and s, respectively, milligram-molecules of water and ammonium sulphate. V_o can therefore be expressed thus:

$$V_o = \frac{V_s}{a + s}.$$

If we substitute this fraction for V_o in the preceding equation, we get — remembering that $E/V_s = \varepsilon$ —:

$$P = \frac{R \cdot T(a + s)}{V_s} \cdot \frac{E}{n\,(a + s)} = R \cdot T \frac{\varepsilon}{n},$$

which is the same expression as that used by us.

The mode of calculation employed by H. N. Morse and his co-operators is also of a similar kind: These inquirers[1]) state the osmotic pressure per gram-molecule of sugar per kilogram of dissolvent, and, as the dissolvent used by Morse is water, a kilogram corresponds here very nearly with one liter.

Provided that our assumption, that the magnitude of the osmotic pressure is proportionate to the concentration ε, is correct, π — which is equal to P/ε — must be found to be a constant quantity, independent of the amount of diffusible matters contained in the solution. As was to be expected, however, the π really found is not constant, but variable according to the contents of ammonium sulphate and hydrogen-ions. Hence, the question arises, how such an interdependence between the composition of the solution and the magnitude of π is to be accounted for. In our inquiries into this question we have been especially considering the under-mentioned three points, which seem to us to deserve notice: —

1) The electrolytical dissociation of albumin salts, with formation of diffusible ions electrostatically fixed; as mentioned in the introduction to this paper, Wo. Pauli as well as B. Moore

[1]) H. N. Morse: The Osmotic Pressure of aqueous Solutions (1914), p. 97.

and his co-operators (see p. 277) in their later works attach particular importance to this point.

2) The effect of an eventual "Donnan counter-pressure".

3) An eventual alteration in the degree of dispersity of the disperse phase, in which respect we have taken into consideration both a diminution of the degree of dispersity (for example by condensation of two or more egg-albumin particles) and an increase of the same (for example by addition of salt to albumin solutions containing little or no salt (compare the view of Moore and Roaf, p. 273)).

Thus, we are here dealing with an aggregate of complicated questions, in which a variety of agencies come into play, and — in spite of the fairly large number of experimental results at our disposal — it has indeed proved impossible to carry through a fairly exhausting treatment of the quantitative aspect of the problem. We have for the present been compelled to limit our researches to the object of substantiating that the alterations found in the value of π can be accounted for in accordance with the views set forth above.

It must be borne in mind that the calculation of π presupposes that not only P, but also the quantity E is known, and the calculation of the latter pre-supposes in its turn the factors y and z to be known. Now, as has been pointed out[1]), the analytical results obtained from a single experiment only enable us to calculate the value of r, whilst those of y and z cannot be determined in this way, but must be estimated by confronting the values found for r in two or more experiments. Thus the quantities y and z, and consequently E and π, are dependent not only on the data established by the actual experiment, but also — though in a less degree — on the results of several such experiments. We have therefore — as will be mentioned in Section F —, on the basis of all the results at our disposal, constructed some curves showing the way in which y and z vary according to the concentrations of ammonium sulphate and hydrogen-ions, and by means of the values derived from these graphical representations we have then made the calculation of E and π. We are certainly aware that our graphs are liable to some modification consequent upon the outcome

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 42 (1916).

of further researches, so that E and π may have to be re-calculated hereafter. But nevertheless we have thought it serviceable to carry out the calculations, the more so as it may help us to ascertain in what directions further investigations will be desirable.

In conclusion, we think it justifiable to emphasise that the experimental results obtained, and exposed in the foregoing subsection (p. 313), cannot be invalidated in the least by any re-calculation; on the contrary, *the figures we have established by direct measurement must form the foundation of any later re-calculation, and we shall therefore set them forth for each experiment.*

In order to render more apparent the manner in which we have proceeded, we may here describe more completely the calculation of π as effected on the basis of the results obtained by Experiment Nr. 61. Our analyses showed (see p. 312) that 100 g. of inner liquid contained:

2.5210 g. ammonia nitrogen (a_b)
1.6319 g. protein „ (p_b),

while 100 g. of outer liquid contained:

2.9169 g. ammonia nitrogen (a_f).

The hydrogen-ion concentration of the inner liquid was

$$13.58 \cdot 10^{\div 6}.$$

As 2.9169 g. of ammonia nitrogen corresponds with

$$2.9169 \cdot 4.7163 = 13.757 \text{ g. } Am_2SO_4,$$

100 g. outer liquid will contain:

13.757 g. Am_2SO_4
86.243 g. water,

so that we find for 100 g. water:

15.951 g. Am_2SO_4 (S).

From Fig. 38 (p. 366) it appears that for $S = 15.951$ we shall have

$$z = 8.69 \text{ for } h = 10 \cdot 10^{\div 6},$$
$$z = 8.67 \text{ for } h = 16 \cdot 10^{\div 6};$$

for $h = 13.58 \cdot 10^{\div 6}$ we therefore use the value $z = 8.68$.

It further appears from Fig. 37 (p. 365) that, if $S = 15.951$, we get

$$y = 0.045 \text{ for } h = 10 \cdot 10^{\div 6},$$
$$y = 0.043 \text{ for } h = 16 \cdot 10^{\div 6};$$

for $h = 13.58 \cdot 10^{\div 6}$ we therefore use the value $y = 0.044$.

100 g. inner liquid will accordingly have the following composition:

$p_b \cdot z = 1.6319 \cdot 8.68 = 14.165$ g. egg-hydrate,

$a_b \cdot 4.7163 \div p_b \cdot y = 2.5210 \cdot 4.7163 \div 1.6319 \cdot 0.044 = 11.818$ g. Am_2SO_4,

the rest being water $= 74.017$ g. water.

Consequently, for 100 g. water we find:

19.137 g. egg-hydrate,
15.967 g. Am_2SO_4 (S_i).

Thus the dispersion medium of the inner liquid contains the same amount of ammonium sulphate ($S_i = 15.967$) as that of the outer liquid ($S = 15.951$), the difference amounting to only about $^1/_{1000}$, that is, less than the analytical error. Hence we conclude, first, that the error due to an unequal distribution of salts is of no consequence, and further, that the values of y and z deduced from the ammonium sulphate amount of the outer liquid may also apply to the inner liquid[1]).

As $P = 66.81$ cm. water-pressure (measured at 20°) (see Table 53 (p. 309) and p. 312), and as V_s (for $S = 15.951$) $= 107.48$[2]), we arrive at:

$$E = \frac{19.137 \cdot 1000}{14.01 \cdot z} = \frac{19137}{14.01 \cdot 8.68} = 157.37$$

$$\varepsilon = \frac{E}{V_s} = \frac{157.37}{107.48} = 1.4642$$

$$\pi = \frac{P}{\varepsilon} = \frac{66.81}{1.4642} = 45.63 \text{ cm. water pressure (measured at } 20^0\text{)}.$$

c. The Elasticity of the Collodion Cap.

As mentioned above, we have considered the alteration undergone by the capillary level in the course of 10 minutes as a measure of the over- or under-pressure. This is, however, only correct if the alteration is exclusively due to the penetration of the dispersion medium through the walls of the collodion cap

[1]) If a material difference should prove to exist between the value of S found for the outer and that for the inner liquid, the composition of the latter must be re-calculated, using the found value first of S for the graphical determination of z and y.

[2]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 158 (Table 32) (1917).

(the velocity of penetration being supposed to be proportionate to the over- or under-pressure, respectively). If, on the contrary, the alteration is partially produced by the expansion or contraction of the cap during the measurement, the results arrived at by the latter will be subject to a greater or smaller error.

We have naturally been attentive to this point, there being no doubt that the cap possesses some elasticity. What is of real interest to us, however, is not the degree of elasticity of the cap, but the question as to whether an expansion or contraction occurs at once to its full extent as soon as the pressure is augmented or diminished respectively, or whether a lingering influence is also exerted. We must bear in mind that the osmometer is left to stand with the cock *C* closed (see Fig. 29, p. 299) during nearly a day and night before the first measurement is proceeded with, and, as the pressure in the osmometer soon reaches a magnitude little short of the osmotic pressure, it follows that even at the outset of the measuring operations the collodion cap has been for some time exposed to practically the same pressure as that at which the said operations are effected. The cap has thus had time enough to assume the volume corresponding with the osmotic pressure, and the alterations of volume caused by minute modifications of pressure from one measurement to the next are quite minimal, and the corresponding after-effects still less.

Although the source of error here referred to cannot probably be of any material consequence, we have nevertheless instituted some experiments with the view of forming an idea as to the order of magnitude of the errors due to the elasticity of the cap. In these experiments, which were made with the caps Nos. 5 and 7, a perfect equilibrium was first established, and, after the osmotic pressure had been determined in the usual manner, it was examined what alteration in the capillary level would be produced by a given under- or over-pressure, regard being had to the condition of the cap before the beginning of the measuring operations, the duration of which was varied.

The result was that an alteration in the counter-pressure of 1 cm immediately produced an alteration in the capillary level of about 6 division lines in the micrometer scale (about 0.2 mm.) for Cap No. 5, and a little more for No. 7. It is thus placed beyond doubt that the caps possess a certain amount of elasticity,

and the inquiry then arises, whether it consists simply in a momentary expansion or contraction, or whether this is followed by a lingering influence. Among the numerous experiments we have made in order to elucidate this question, one may be instanced as representative of the rest. It was made with Cap No. 5, and at the outset the system was in absolute equilibrium, with a total counter-pressure of 71.10 cm. water-pressure. After the counter-pressure had been reduced by about 0.8 cm., the capillary level was observed at intervals of five minutes. The result was as follows:

Time elapsed since diminution of counter-pressure, minutes.	Ascension of Capillary Level in micrometer divisions; in total	per 10 minutes.	Magnitude of Under-pressure in cm. water-pressure.
0	—	—	0.82
5	5.2	10.4	0.80
10	9.9	9.9	0.79
15	14.8	9.9	0.78
20	19.2	9.6	0.76
25	23.4	9.4	0.75
30	27.5	9.2	0.74
35	31.1	8.9	0.73
40	34.7	8.7	0.72.

It appears from this table that the ascension of the capillary level per ten minutes is getting less and less in process of time. This is no doubt due in some measure to the circumstance that the counter-pressure is gradually diminished by that ascension (33.8 micrometer divisions = 1 mm.); but the lingering influence alluded to must also be taken into account. When the measuring process is commenced, the counter-pressure is diminished by 0.82 cm. water-pressure, and the cap consequently contracts; this contraction is mainly momentary, yet continued in some degree further on. Thus, the rise observed in the capillary level is not caused solely by outer liquid having diffused into the tube, but also by the contraction of the cap, and of course the phenomenon manifests itself most intensely in the first minutes after the diminution of the counter-pressure.

In order to clear up the importance to be attached to this source of error in the determination of the zero, or, in other

words, of the magnitude of the osmotic pressure (see p. 301 and Fig. 32 (p. 311)), we have carried out a number of experiments, which have shown the following points to be of particular moment:

1) The first reading off of the capillary level must not take place immediately after the alteration of the counter-pressure, but only after some minutes have elapsed, so that the secondary effect will be of little or no consequence.

2) In each measurement the same space of time must, as far as possible, be used between the first and the last reading of the capillary level, in order that the after-effect may be of practically the same consequence in each particular instance.

3) The state of equilibrium should be, as far as possible, used as starting point. It is true that a diminution of the counter-pressure will in this case produce a higher ascension of the capillary level than that produced by the diffusion through the walls of the cap, while an increase of the counter-pressure will have the opposite effect, resulting in a different value of α (see p. 301 and Fig. 32, p. 311); but the zero will remain unaltered.

If the points adverted to are taken into due consideration, the determination of the zero will not be affected to any appreciable degree. if at all, by the elasticity of the cap. But the neglect of those precautions will influence the results in a more or less marked degree, the lingering influence repeatedly referred to being particularly apt to make itself felt if the duration of each measuring process is very different from the others, or if, instead of starting always from the state of equilibrium, the manipulations are commenced at one time at a high over-pressure, but the next time at a marked under-pressure, or conversely.

When carrying out our first experiments, the importance of the source of error here discussed was not clear before us (see for example Table 53, p. 308), nor is it always possible to arrange everything in such a manner as to preclude any error due to the elasticity of the cap. Some of the results we have arrived at may therefore possibly demand correction for errors coming from this source. We think, however, that such minute errors are of no practical consequence at all. Indeed, the control experiments related above show that the greatest error entailed in the zero determination when the observations of the alterations undergone by the capillary level were combined in the manner

most contrary to the rules laid down amounted to only 0.24 cm. water-pressure. *As we have never — not even in our very earliest experiments — offended so grossly against the said rules, we think we may safely, even with regard to our first experiments, disregard the source of error in question.*

f. Is the Collodion Cap absolutely impermeable to Egg-albumin?

Finally, we propose to deal with a question to which attention was drawn some years ago by A. von Antropoff[1]). This *savant* showed that, if the membrane used is not perfectly impermeable to the non-diffusible matter, the maximum pressure in a measurement of osmotic pressure carried out by the current method will prove inferior, sometimes far inferior, to the real osmotic pressure, even if during the course of the experiment the non-diffusible matter has only penetrated the membrane in so slight quantity that the concentration in the outer liquid is extremely low. The reason is traced back by Antropoff to the fact that the maximum pressure with a but partially semi-permeable membrane is really not the pressure just sufficient to prevent the dissolvent penetrating the membrane, but that pressure at which the volume of the dissolvent entering is equal to the quantity, by volume, of non-diffusible matter passing through the membrane in the opposite direction. Accordingly, the maximum pressure will not correspond with, but be inferior to, the osmotic pressure.

The source of error resulting from the circumstance here referred to will also be sure to make its influence felt in the mode of proceeding adopted by us, — unless the permeability of the collodion to egg-albumin can be ignored. The fact is that a sufficient permeability will have the effect that the counter-pressure at which no alteration is observable in the capillary level does not correspond with the pressure at which the dispersion medium neither immigrates into nor emigrates from the cap, but simply indicates that the volume of dispersion medium entering the cap is equal to that of egg-albumin coming out. We must therefore try to form an estimate of the influence which may be exerted on our experimental results by the source of error in question.

Let δ signify the quantity, measured in cubic millimeters,

[1]) Zeitschr. physik. Chem., **76**, 721 (1911).

of dispersion medium passing through the cap in unit of time (10 minutes) per one cm. over- or under-pressure, and $\triangle$ the amount of egg-hydrate, in cubic millimeters, which under the conditions of the experiment passes into the outer liquid in the same period of time. The volume of the solution contained in the capillary tube will then be increased, in unit of time, by

$$(P \div p)\, \delta \div \triangle$$

cubic millimeters; we thus arrive at the following equation (the same designations being used as explained in the subsections *a* and *c*):

$$(P \div p)\, \delta \div \triangle = \frac{v \cdot s}{33.8},$$

where s means the sectional area, in square millimeters, of the capillary tube, while 33.8 is the number of divisions of the micrometer scale per mm.

If v is equal to 0, we have reckoned p equal to P, whereas the equation now obtained gives:

$$(P \div p)\, \delta \div \triangle = 0$$

$$p = P \div \frac{\triangle}{\delta}.$$

The order of magnitude of $\triangle$ can be estimated from the determinations of the coagulable egg-albumin existing in the outer liquid at the completion of the experiment. At that moment we generally found between 0.05 and 0.20 mg. of "coagulable nitrogen" in 100 cc. of outer liquid, so that the whole amount of that liquid (about 250cc.) contained 0.13—0.50 mg. Only in exceptional cases did it reach 1 mg. Such a high amount may, we believe, be considered as quite abnormal and attributable to a deficiency in the experimental arrangement: the ground part may not have been tight-fitting, or when placing the capillary tube in the same, a little inner liquid may have come out on the outside of the cap. If we reckon with 1 mg. of coagulable nitrogen in the whole of the outer liquid, a simple calculation will show that this corresponds with about 6.7 cubic millimeters of egg-hydrate, supposing that the factor by which the weight of the protein nitrogen is to be multiplied to give the weight of the egg-hydrate is put equal to 8.4 and the specific gravity of the egg-hydrate equal to 1.26. If, further, the duration of the experiment is reckoned at 3 days — the shortest duration of any of our experiments on this subject —, we find for the amount of egg-hydrate which has diffused out in the unit of time:

$$\Delta = \frac{6.7}{6 \cdot 24 \cdot 3} = \text{about } 0.015 \text{ cubic millimeters.}$$

Since we have found:

$$\delta = \frac{\frac{v \cdot s}{33.8} + \Delta}{P \div p},$$

it is thus possible to calculate δ if Δ is known.

By way of example we may take some of the measurements in Experiment No. 61 (Table 53, p. 308), where v has its highest numerical value, and where δ can therefore be determined with the greatest degree of exactness. The apparatus used in that experiment was No. II, the capillary tube of which had a sectional area, s, of 3.68 square millimeters; $\frac{s}{33.8}$ will thus be $= 0.109$ square millimeters.

Thus, since $\Delta = 0.015$ cubic millimeters, and putting $P \div p$ equal to $a \cdot v$ (Table 53, third column from the right), we arrive at the following values of δ:

Measurement No. 1: $\delta = \frac{\div 13.0 \cdot 0.109 + 0.015}{\div 7.54} = 0.19$

„ „ 2: $\delta = \frac{\div 7.1 \cdot 0.109 + 0.015}{\div 4.12} = 0.18$

„ „ 7: $\delta = \frac{+ 1.2 \cdot 0.109 + 0.015}{+ 0.70} = 0.21$

„ „ 13: $\delta = \frac{+ 1.1 \cdot 0.109 + 0.015}{+ 0.70} = 0.19.$

Mean value of δ: 0.19 cubic millimeters.

The expression:

$$p = P \div \frac{\Delta}{\delta},$$

may now in the present case be written:

$$p = P \div \frac{0.015}{0.19} = P \div 0.08.$$

Hence, it will be seen that *the error made by putting $p = P$ if $v = 0$ is really of no account, in spite of the circumstance that the cap was supposed to have an extraordinary permeability to egg-hydrate.* We may therefore in the following leave this source out of consideration, since it is of no consequence with the collodion caps used in our experiments.

B. Does an Egg-albumin solution of given composition show a constant Osmotic Pressure?

In dealing with this question we aimed at ascertaining the extent of the variation to which the osmotic pressure is subject in the course of conservation of an egg-albumin solution, on the one hand, and, on the other, the difference of pressure exhibited by different preparations of egg-albumin. Our experiments having thus for their object to determine the magnitude of the osmotic pressure peculiar to each of several samples of egg-albumin as well as the variations undergone by that pressure in one and the same sample in the progress of time, it was of course necessary to effect the measurements, as far as possible, always under identical conditions, that is, at the same concentration of ammonium sulphate, hydrogen-ions and egg-albumin, and at the same temperature. Now it will be easily understood that it is an exceedingly difficult matter, if not one of sheer impossibility, to provide exactly the same experimental conditions in research-work carried out at intervals of several months with materials (solutions of egg-albumin and ammonium sulphate) of different composition and on experimental principles pre-supposing a state of equilibrium brought about by a diffusion between the inner and outer liquid. Fortunately, it is not indispensable to our present purpose to adhere rigorously to those exigencies in every detail, since it is possible, by the aid of slight corrections applied to the results of our measurements, to take into account any variations that may have occurred in the experimental circumstances. The fact is, that — as will be explained in the following chapters — the dependence of osmotic pressure on the circumstances admits of being expressed graphically, so that the required corrections can be procured without any difficulty.

In Table 54 we have grouped together a set of measurements which seem to throw some light on the question dealt with in this chapter. Indeed, some of the experiments tabulated were instituted precisely for this purpose, whilst others are chosen from several series carried out with entirely different ends in view. The contents of the table have thereby gained in abundance and variety; but, on the other hand, it has sometimes been necessary to apply rather considerable corrections, because the

circumstances under which the experiments were carried out differed considerably from those adopted as normal for this series, *viz.* an ammonium sulphate concentration corresponding with $S =$ 15.6, a hydrogen-ion concentration in inner liquid of $13 \cdot 10^{\div 6}$, and an albumin concentration corresponding with $\varepsilon = 1.2$ to 1.8.

The numerical results set forth in the table need no further explanation than that given above of the way in which they were obtained and the osmotic pressure measured (see p. 307 and p. 318). It is only necessary to add a few remarks on the correction of the value found for π (the last three vertical columns of the table).

The third column from the right gives the correction which has to be added to π to give the value that π would have had if S had under otherwise unaltered circumstances had the value 15.6; this correction can be found with the help of Fig. 33 (p. 338). For example, in the first of the experiments set forth in the table (No. 57) we have $S = 14.719$; from the middle curve of Fig. 33 (corresponding with $h = 13 \cdot 10^{\div 6}$) it is seen that for $S = 14.719$ we have $\pi = 48.06$, while if $S = 15.6$ we get $\pi = 46.80$; hence, the correction will be $46.80 \div 48.06 = \div 1.26$. This is the way in which all the corrections in this column were found. Since the three curves which in Fig. 33 bring out the mutual dependence of S and π at the hydrogen-ion concentrations $25 \cdot 10^{\div 6}$, $13 \cdot 10^{\div 6}$ and $5 \cdot 10^{\div 6}$ are approximately parallel, at any rate as far as the portions here considered are concerned, it appears legitimate to determine the corrections by means of the middle curve alone, which corresponds with $h = 13 \cdot 10^{\div 6}$, even if the hydrogen-ion concentration differs considerably from this value.

The corrections in the last column but one were found in essentially the same way, only with this difference that Fig. 34 (p. 346) was used[1]).

Finally, the corrected value of π was determined by the addition of the above corrections to the value found by experiment, and it is of course the corrected values thus obtained and stated in the last column of the table which are to be compared

[1]) The sixth column from the right shows that ε varied in the different experiments from about 1.2 to 1.8; yet, as will appear by a series of experiments described in Section E (p. 347), this variation does not necessitate any correction of π.

TABLE 54.

Osmotic Pressure of some Egg-albumin soluti

No. of experiment.	Egg-albumin solution used.		Eksperimenter; No. of osmometer and No. of cap.	Found on analysis				Ammonium sulphate content of *outer* liquid in σ
				in 100 g. *outer* liquid	in 100 g. *inner* liquid		Hydrogen-ion concentration of *inner* liquid, h.	
	Mark.	Age, in days.		Ammonia nitrogen in g. (a_f)	Ammonia nitrogen in g. (a_b)	Protein nitrogen in g. (p_b)	$h \cdot 10^6$	S
57	I. D. Æ. 1	15	Chr. I 3	2.7205	2.2693	1.9100	12.16	14.7
58	»	16	Chr. II 6	2.5848	2.1772	1.9090	13.15	13.8
59	»	20	Chr. II 6	2.4761	2.0933	1.8698	12.71	13.2
61	»	24	Chr. II 6	2.9169	2.5210	1.6319	13.58	15.9
87	»	175	Golds. II 6	2.8636	2.4884	1.6046	11.14	15.61
88	»	175	Golds. III 3	2.8559	2.4637	1.6534	10.96	15.5
89	»	182	Golds. II 6	2.8632	2.4778	1.6315	11.51	15.61
90	»	182	Golds. III 3	2.8659	2.4754	1.6429	10.45	15.62
137	I. D. Æ. 7	18	Golds. II 6	2.8319	2.4648	1.5064	13.43	15.41
138	»	18	Golds. III 3	2.8321	2.3651	1.9706	13.84	15.41
163	»	407	Pal. IX 5	2.8382	2.4432	1.6775	14.45	15.45
164	»	407	Pal. VII 7	2.8324	2.4468	1.6547	13.06	15.41
103	D. Æ. 4	80	Golds. II 6	2.9621	2.5983	1.4847	10.50	16.23
110	»	129	Golds. III 3	2.5747	2.2663	1.4299	11.59	13.82
111	»	174	Golds. II 6	2.5653	2.2577	1.4142	12.42	13.76
127	»	216	Golds. II 6	2.8880	2.5519	1.4061	4.85	15.76
165	»	690	Pal. IX 5	2.6501	2.3270	1.4927	4.54	14.28
166	»	690	Pal. VII 7	3.3234	2.9084	1.4732	4.61	18.58

nearly like composition.

Values of z, y and V_s corresponding with ound values of h and S.			The *inner* liquid then contains per 100 g. water			$\varepsilon = \frac{E}{V_s}$	*Osmotic pressure* in cm. water pressure				
			Ammonium sulphate in g.	Egg-hydrate in g.	Milligr.-equival. protein nitrogen, $E = \frac{E_i \cdot 1000}{14.01 \cdot z}$		P (measured).	$\pi = \frac{P}{\varepsilon}$.	Correction, in cm., to be added to π in order to refer the measurement to		*Corrected* value of π (corresponding to $S = 15.6$ and $h = 13 \cdot 10^{+6}$).
z	y	V_s							$S = 15.6$.	$h = 13 \cdot 10^{+6}$.	
ig. 38 366).	(Fig. 37 p. 365).	(Tab. 32 p. 158).	S_i	E_i							
3.69	0.045	106.87	14.587	22.804	187.30	1.7526	86.16	49.16	÷ 1.26	÷ 0.40	47.50
3.69	0.045	106.45	13.908	22.660	186.12	1.7484	86.29	49.35	÷ 2.45	+ 0.05	46.95
3.70	0.046	106.13	13.234	21.998	180.47	1.7005	84.18	49.50	÷ 3.25	÷ 0.12	46.13
3.68	0.044	107.48	15.967	19.137	157.37	1.4642	66.81	45.63	+ 0.50	+ 0.27	46.40
3.69	0.044	107.31	15.681	18.744	153.95	1.4346	68.00	47.40	+ 0.01	÷ 0.92	46.49
3.69	0.044	107.29	15.586	19.394	159.29	1.4847	69.60	46.88	÷ 0.02	÷ 1.00	45.86
3.69	0.044	107.31	15.651	19.106	156.93	1.4624	69.28	47.37	+ 0.01	÷ 0.70	46.68
3.69	0.044	107.32	15.654	19.262	158.21	1.4742	72.26	49.02	+ 0.02	÷ 1.25	47.79
3.69	0.044	107.21	15.342	17.376	142.72	1.3312	61.69	46.34	÷ 0.30	+ 0.20	46.24
3.69	0.044	107.21	15.414	23.849	195.88	1.8271	85.15	46.60	÷ 0.30	+ 0.38	46.68
3.69	0.044	107.23	15.477	19.706	161.86	1.5095	70.95	47.00	÷ 0.22	+ 0.60	47.38
3.69	0.044	107.22	15.464	19.391	159.27	1.4855	69.99	47.12	÷ 0.30	0.00	46.82
3.69	0.044	107.63	16.272	17.224	141.47	1.3144	60.78	46.24	+ 0.95	÷ 1.25	45.94
3.70	0.045	106.42	13.811	16.170	132.66	1.2466	63.19	50.69	÷ 2.50	÷ 0.68	47.51
3.70	0.046	106.40	13.724	15.956	130.91	1.2304	62.09	50.46	÷ 2.58	÷ 0.30	47.58
3.69	0.044	107.39	15.795	16.119	132.39	1.2328	62.77	50.92	+ 0.20	÷ 5.27	45.85
3.69	0.045	106.65	14.330	17.042	139.98	1.3125	70.10	53.41	÷ 1.92	÷ 5.55	45.94
3.68	0.043	108.81	18.562	17.383	142.94	1.3137	63.02	47.97	+ 4.90	÷ 5.42	47.45

Mean: 46.7

to furnish a reliable answer to the question with which we are concerned. Now, an inspection of the last column will show a very satisfactory accordance between the corrected values of π, inasmuch as the deviation from the mean value does only in a single instance exceed 1 cm., while being generally much smaller. There can be no doubt that this deviation falls within the limits of the experimental errors, and we are therefore warranted in concluding that the egg-albumin solutions examined under the "Normal experimental circumstances" chosen for the series considered do exhibit the same osmotic pressure.

If it is now asked, of what nature are the above egg-albumin solutions, information can be obtained from the first columns of the table. The second column shows that the experiments were made on two samples of non-dialysed albumin solution (marked I. D. Æ. 1 and I. D. Æ. 7, respectively) and one sample of dialysed albumin solution (marked D. Æ. 4), all the three samples being examined at very different ages. As was mentioned in a preceding paper[1]), our egg-albumin solutions with admixtures of toluol are kept in ice in an ice-safe, by which means it is possible to prevent the albumin from being decomposed by the action of micro-organisms. But it is not possible to prevent a smaller or greater part of the albumin from being separated in a denaturated condition. It is true that as far as the non-dialysed samples are concerned, which contain ammonium sulphate in abundance, the denaturation, even after many months' conservation, does not attain any appreciable extent; but the case is different with dialysed solutions, which contain little or no ammonium sulphate.

Thus, for example, the dialysed egg-albumin solution in Table 54 showed after a lapse of 80 days an appreciable precipitate, which was then found to increase gradually. Yet after 216 days the precipitate did not amount to more than a few per cent of the total quantity of albumin. But at the end of 690 days it had reached a very considerable amount, a quantitative determination showing that as much as about 58 % of the whole amount of albumin had separated in a denaturated state. Therefore, to obtain, as far as possible, the same albumin concentration in the experiments Nos. 165 and 166 as in our other

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**. 36 (1916).

experiments, it was necessary, after filtering off the precipitate, to dissolve a sufficient dose of solid ammonium sulphate in the filtrate, instead of mixing — as we did in the other experiments — the albumin solution with a sufficient portion of ammonium sulphate solution. Now, the numerical results of these two experiments prove the osmotic pressure of the non-denaturated albumin remaining in dissolution to be quite normal, so that no products intermediate between the genuine and the denaturated albumin seem to be formed in the denaturation process, at any rate none that manifests itself by alterations in the osmotic pressure of the solution.

In one respect, however, the solution was found to have altered at the same time with the denaturation of the albumin: the hydrogen-ion concentration, which before denaturation amounted to $10—12 \cdot 10^{\div 6}$, had gone down to $4—5 \cdot 10^{\div 6}$ (see the seventh column). Experiment No. 127, which was made on the albumin solution before any material denaturation had taken place, forms part of a series of experiments having for their object to ascertain how far the osmotic pressure was influenced by the hydrogen-ion concentration (see Section D). The low hydrogen-ion concentration in Experiment No. 127 of this series was due to an admixture of ammonia. The said experiment is included in Table 54 only for the sake of comparison with Experiments Nos. 165 and 166, with which it has in common — in contradistinction from the rest — the low concentration of hydrogen-ions[1]).

[1]) Although we have not as yet effected any further investigation into the interrelations existing between the denaturation of albumin and the hydrogen-ion concentration of the solution, yet we may here refer to certain facts pointed out by us in recent papers (Comptes-rendus du Laboratoire de Carlsberg, **12**, 60 (1916) and **12**, 196 (1917)), which seem to show that the denaturation gives rise to a formation of ammonia. The amount of ammonia separated is, however, too insignificant to be the only cause of such a considerable alteration in the hydrogen-ion concentration. But it is of course possible that other substances are split off as well. If they are, our experiments go to show that they must be diffusible, since they do not manifest themselves in the measurement of the osmotic pressure.

It is to be remarked, however, that the outer liquids did not show any very large amount of either "coagulable" or "non-coagulable" protein-nitrogen (100 cc. of outer liquid contained in No. 165: 0.05 mg., in No. 166: 0.04 mg. of "coagulable nitrogen"; in No. 165: 0.18 mg. and in No. 166: 0.21 mg. "non-coagulable nitrogen").

After all, the diminution of the hydrogen-ion concentration may therefore be only partially, if at all, due to the circumstance referred to. It may

In conclusion, we may mention that the experiments shown in the table were executed — as stated in the third column — by three operators (Messieurs I. A. Christiansen, S. Goldschmidt and S. Palitzsch), who carried out the osmotic measurements by means of five different osmometers and four different collodion caps. A biassed judgment of things is therefore out of the question.

Accordingly we believe to be warranted in concluding, that *an egg-albumin solution of a given composition will invariably show one and the same osmotic pressure.*

C. The dependence of Osmotic Pressure on Ammonium sulphate concentration.

The influence exerted by the ammonium sulphate concentration on the magnitude of the osmotic pressure of an albumin solution is shown in the Tables 55, 56 og 57, in each of which we have grouped together a series of experiments where the amount of ammonium sulphate was varied, while the other experimental conditions were kept practically constant.

The tables are elaborated on exactly the same principle as Table 54, and therefore need no further explanation. In the ninth vertical column of each is stated the value of S, the variable quantity of each series. The hydrogen-ion concentration is different from one series to another, amounting to $13 \cdot 10^{\div 6}$, $25 \cdot 10^{\div 6}$ and $5 \cdot 10^{\div 6}$, respectively. Since — as shown by the eight column — the hydrogen-ion concentration of the inner liquid is not exactly equal to the normal concentration chosen for the series, the value found for π has been corrected by means of Fig. 34 (p. 346), so that the *corrected* value for π, as given in the last column, represents the magnitude which π would have if the hydrogen-ion concentration were that chosen for the series, the experimental circumstances being otherwise unaltered [1]).

perhaps be accounted for, in part at least, by the fact that the denaturated albumin has fixed more sulphuric acid than ammonia. It must also be remembered that the glass, on being left to stand for such a long time, may give off an appreciable amount of alkaline matter.

[1]) It appears from the fifth column from the right that ε has in the different experiments been varying only from about 1.2 to 1.5, a variation which —

The magnitude of the necessary corrections has in every case been estimated from the full-drawn curve in Fig. 34, although this curve pre-supposes an ammonium sulphate concentration corresponding with $S = 15.6$. The fact is that, as will be seen in the next chapter, this is the only ammonium sulphate concentration at which the experimental materials at our disposal are sufficiently ample to serve as the basis of an adequate graphical representation of the influence exerted by the hydrogen-ion concentration. On the other hand, the dotted curve in Fig. 34, where the ammonium sulphate concentration corresponds with $S = 4.3$, but for the exact construction of which we have not a sufficient number of experiments at our command, is nearly parallel to the main curve, and we therefore consider that the error inherent to the small corrections relative to Tables 55, 56 and 57 is of no material consequence.

On this occasion we feel ourselves called upon to confess that we are fully aware of the fundamental incorrectness committed in using Fig. 34 for the estimation of the corrections to be applied to the experimental results set forth above, whereas further on (see p. 341) the curves drawn on the basis of the results exposed in Tables 55, 56 and 57 (Fig. 33) are employed as artificial curves for the construction of Fig. 34. But we see no other way out of the difficulty; neither do we consider the errors to which our proceeding may give rise to be of any material consequence. In fact, the corrections are determined in either case and for each table from one and the same little portion of the curve in question, and in that minute section the form of the curve is determined not solely by the experimental results on which the section is based, but by the whole of the results expressed in the curve.

We therefore consider that even though the corrections calculated by our procedure are only approximately exact, yet the corrected values of π afford a more reliable account of the state of things than do the not corrected values. Still, it must be borne in mind that *the latter form the foundation of the whole system, the basis to which we shall have to resort in the case of an eventual recalculation of our results.*

The experimental results put together in Tables 55, 56 and 57 are graphically reproduced in Fig. 33, the corrected value of π (the last colum of the tables) being plotted as ordinate against S (ninth column) as abscissa.

As stated in Fig. 33, each of the three curves corresponds with a particular hydrogen-ion concentration. It will be seen at once that the middle curve, corresponding with the concentration $13 \cdot 10^{-6}$, is the one which has been determined with the greatest degree of exactness, the measurements on which it is based being considerably more numerous than those underlying

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as mentioned in the footnote on p. 327 — does not necessitate any correction of π.

TABLE 55.

Dependence of Osmotic Pressur

No. of experiment.	Egg-albumin solution used.		Eksperimenter; No. of osmometer and No. of cap.	Found on analysis				
	Mark.	Age, in days.		in 100 g. *outer* liquid	in 100 g. *inner* liquid		Hydrogen-ion concentration of *inner* liquid	Ammonium sulphate content of *outer* liquid
				Ammonia nitrogen in g. (a_f)	Ammonia nitrogen in g. (a_b)	Protein nitrogen in g. (p_b)	h	S
98	D. Æ. 4	59	Golds. III 3	4.1399	3.6342	1.4392	10.33	24.2
104	»	80	Golds. III 3	4.0020	3.5101	1.4647	10.21	23.2
62	I. D. Æ. 1	26	Chr. I 3	3.5104	3.0188	1.6388	12.22	19.8
112	D. Æ. 4	174	Golds. III 3	3.3241	2.9358	1.3928	11.64	18.5
97	»	58	Golds. II 6	3.2861	2.8746	1.4870	10.33	18.3
103	»	80	Golds. II 6	2.9621	2.5983	1.4847	10.50	16.2
61	I. D. Æ. 1	24	Chr. II 6	2.9169	2.5210	1.6319	13.58	15.9
90	»	182	Golds. III 3	2.8659	2.4754	1.6429	10.45	15.62
87	»	175	Golds. II 6	2.8636	2.4884	1.6046	11.14	15.61
89	»	182	Golds. II 6	2.8632	2.4778	1.6315	11.51	15.61
88	»	175	Golds. III 3	2.8559	2.4637	1.6534	10.96	15.56
163	I. D. Æ. 7	407	Pal. IX 5	2.8382	2.4432	1.6775	14.45	15.45
164	»	407	Pal. VII 7	2.8324	2.4468	1.6547	13.06	15.41
137	»	18	Golds. II 6	2.8319	2.4648	1.5064	13.43	15.41
110	D. Æ. 4	129	Golds. III 3	2.5747	2.2663	1.4299	11.59	13.82
111	»	174	Golds. II 6	2.5653	2.2577	1.4142	12.42	13.76
105	»	86	Golds. II 6	2.1341	1.8792	1.4744	10.86	11.19
96	»	52	Golds. III 3	1.6859	1.4813	1.5384	10.52	8.63
146	D. Æ. 7	72	Golds. III 3	0.8816	0.7779	1.4823	13.37	4.33
95	D. Æ. 4	52	Golds. II 6	0.8581	0.7590	1.5433	10.40	4.21
94	»	46	Golds. III 3	0.5312	0.4736	1.5835	11.27	2.57
93	»	46	Golds. II 6	0.3579	0.3228	1.5815	10.12	1.71
82	D. Æ. 3	90	Chr. II 6	0.1881	0.1688	1.7714	14.45	0.89
92	D. Æ. 4	32	Golds. III 3	0.1786	0.1626	1.5614	11.32	0.84
81	D. Æ. 3	90	Chr. III 3	0.09363	0.08617	1.7781	15.67	0.444
91	D. Æ. 4	32	Golds. II 6	0.09219	0.08690	1.5997	10.84	0.437

monium sulphate concentration ($h = 13 \cdot 10^{\div 6}$).

Values of z, y and V_s corresponding with und values of h and S.			The *inner* liquid then contains per 100 g. water			$\varepsilon = \frac{E}{V_s}$.	*Osmotic pressure* in cm. water pressure.			
z	y	V_s	Ammonium sulphate in g. S_i	Egg-hydrate in g. E_i	Milligr.-equival. protein nitrogen E $E = \frac{E_i \cdot 1000}{14.01 \cdot z}$.		P (measured).	$\pi = \frac{P}{\varepsilon}$.	Correction, in cm., to be added to π in order to refer the measurem. to $h = 13 \cdot 10^{\div 6}$	*Corrected* value of π (corresponding with $h = 13 \cdot 10^{\div 6}$).
ig. 38 366).	(Fig. 37 p. 365).	(Tab. 32 p. 158).								
3.69	0.040	111.75	24.260	17.763	145.90	1.3056	39.63	30.35	÷ 1.35	29.00
3.69	0.040	111.23	23.307	17.983	147.70	1.3279	43.94	33.09	÷ 1.40	31 69
3.69	0.042	109.45	19.792	19.892	163.38	1.4927	59.25	39.69	÷ 0.36	39.33
3.69	0.043	108.81	18.602	16.331	134.14	1.2328	52.97	42.97	÷ 0.62	42.35
3.69	0.043	108.69	18.337	17.561	144.24	1.3271	58.19	43.85	÷ 1.35	42.50
3.69	0.044	107.63	16.272	17.224	141.47	1.3144	60.78	46.24	÷ 1.25	44.99
3.68	0.044	107.48	15.967	19.137	157.37	1.4642	66.81	45.63	+ 0.27	45.90
3.69	0.044	107.32	15.654	19.262	158.21	1.4742	72.26	49.02	÷ 1.25	47.77
3.69	0.044	107.31	15.681	18.744	153.95	1.4346	68.00	47.40	÷ 0.92	46.48
3.69	0.044	107.31	15.651	19.106	156.93	1.4624	69.28	47.37	÷ 0.70	46.67
3.69	0.044	107.29	15.586	19.394	159.29	1.4847	69.60	46.88	÷ 1.00	45.88
3.69	0.044	107.23	15.477	19.706	161.86	1.5095	70.95	47.00	+ 0.60	47.60
3.69	0.044	107.22	15.464	19.391	159.27	1.4855	69.99	47.12	0.00	47.12
3.69	0.044	107.21	15.342	17.376	142.72	1.3312	61.69	46.34	+ 0.20	46.54
3.70	0.045	106.42	13.811	16.170	132.66	1.2466	63.19	50.69	÷ 0.68	50.01
3.70	0.046	106.40	13.724	15.956	130.91	1.2304	62.09	50.46	÷ 0.30	50.16
3.72	0.049	105.15	11.220	16.409	134.31	1.2773	67.93	53.18	÷ 1.06	52.12
3.75	0.052	103.93	8.672	16.904	137.89	1.3268	73.13	55.12	÷ 1.25	53.87
3.62	0.046	101.97	4.306	15.279	126.51	1.2407	69.17	55.75	+ 0.18	55.93
3.67	0.050	101.92	4.215	16.098	132.53	1.3003	73.71	56.69	÷ 1.30	55.39
3.24	0.036	101.20	2.568	15.391	133.56	1.3198	75.37	57.11	÷ 0.80	56.31
7.84	0.028	100.84	1.716	14.397	131.07	1.2998	75.65	58.20	÷ 1.45	56.75
7.48	0.017	100.50	0.891	15.410	147.06	1.4633	79.49	54.32	+ 0.60	54.92
7.33	0.018	100.48	0.842	13.033	126.92	1.2631	70.69	55.97	÷ 0.78	55.19
7.15	0.012	100.32	0.4431	14.629	146.04	1.4557	79.54	54.64	+ 1.08	55.72
5.92	0.014	100.31	0.4375	12.502	128.95	1.2855	73.10	56.87	÷ 1.06	55.81

TABLE 56. **Dependence of Osmotic Pressure**

No. of experiment.	Egg-albumin solution used. Mark.	Egg-albumin solution used. Age, in days.	Eksperimenter; No. of osmometer and No. of cap.	Found on analysis: in 100 g. *outer* liquid. Ammonia nitrogen in g. (a_f).	Found on analysis: in 100 g. *inner* liquid. Ammonia nitrogen in g. (a_b).	Found on analysis: in 100 g. *inner* liquid. Protein nitrogen in g. (p_b).	Hydrogen-ion concentration of *inner* liquid, h. $h \cdot 10^6$	Ammonium sulphate content of *outer* liquid, in g. S
117	D. Æ. 4	191	Golds. II 6	3.6570	3.2233	1.3968	24.83	20.84
114	»	180	Golds. III 3	3.5442	3.1277	1.3862	23.50	20.07
108	»	96	Golds. III 3	3.3339	2.9207	1.4400	22.49	18.65
116	»	185	Golds. III 3	2.9205	2.5654	1.3985	25.41	15.97
113	»	180	Golds. II 6	2.5399	2.2425	1.4071	23.07	13.60
115	»	185	Golds. II 6	2.0750	1.8344	1.4404	24.49	10.84
107	»	96	Golds. II 6	1.7510	1.5445	1.4266	22.59	9.00
129	»	224	Golds. II 6	0.8837	0.7829	1.4612	23.33	4.34
122	»	205	Golds. III 3	0.5253	0.4701	1.5212	25.06	2.54
121	»	205	Golds. II 6	0.3576	0.3186	1.4924	25.23	1.71
84	D. Æ. 3	97	Chr. III 3	0.1887	0.1666	1.8041	26.79	0.89
120	D. Æ. 4	200	Golds. III 3	0.1770	0.1597	1.4803	25.83	0.84
83	D. Æ. 3	97	Chr. II 6	0.09237	0.08201	1.7908	29.51	0.43
119	D. Æ. 4	200	Golds. II 6	0.08840	0.08010	1.4865	26.36	0.41

TABLE 57. **Dependence of Osmotic Pressure**

No. of experiment.	Egg-albumin solution used. Mark.	Egg-albumin solution used. Age, in days.	Eksperimenter; No. of osmometer and No. of cap.	Found on analysis: in 100 g. *outer* liquid. Ammonia nitrogen in g. (a_f).	Found on analysis: in 100 g. *inner* liquid. Ammonia nitrogen in g. (a_b).	Found on analysis: in 100 g. *inner* liquid. Protein nitrogen in g. (p_b).	Hydrogen-ion concentration of *inner* liquid, h. $h \cdot 10^6$	Ammonium sulphate content of *outer* liquid, in g. S
167	D. Æ. 4	701	Pal. IX 5	3.5673	3.1509	1.4048	3.78	20.22
166	»	690	Pal. VII 7	3.3234	2.9084	1.4732	4.61	18.58
127	»	216	Golds. II 6	2.8880	2.5519	1.4061	4.85	15.76
165	»	690	Pal. IX 5	2.6501	2.3270	1.4927	4.54	14.28
106	»	87	Golds. III 3	2.5593	2.2587	1.4675	7.73	13.72
168	»	701	Pal. VII 7	1.9407	1.7324	1.6089	4.06	10.07
133	»	235	Golds. II 6	0.8889	0.7918	1.4386	5.50	4.37

ımonium sulphate concentration ($h = 25 \cdot 10 \div 6$).

'alues of z, y and V_s corresponding with ınd values of h and S.			The *inner* liquid then contains per 100 g. water			$\varepsilon = \frac{E}{V_s}$.	*Osmotic pressure* in cm. water pressure.			
z	y	V_s	Ammonium sulphate in g. S_i	Egg-hydrate in g. E_i	Milligr.-equival. protein nitrogen, $E = \frac{E_i \cdot 1000}{14.01 \cdot z}$		P (measured).	$\pi = \frac{P}{\varepsilon}$.	Correction in cm., to be added to π in order to refer the measurement to $h = 25 \cdot 10 \div 6$.	*Corrected* value of π (corresponding with $h = 25 \cdot 10 \div 6$).
g. 38 366).	(Fig. 37 p. 365).	(Tab. 32 p. 158).								
.68	0.041	109.97	20.823	16.670	137.08	1.2465	37.02	29.70	÷ 0.05	29.65
67	0.041	109.57	20.050	16.398	135.00	1.2321	42.03	34.11	÷ 0.41	33.70
67	0.042	108.85	18.584	16.917	139.27	1.2795	49.62	38.78	÷ 0.67	38.11
66	0.042	107.49	15.874	15.963	131.57	1.2240	52.02	42.50	+ 0.10	42.60
66	0.042	106.32	13.606	15.764	129.93	1.2221	53.45	43.74	÷ 0.51	43.23
65	0.042	104.98	10.883	15.781	130.22	1.2404	60.24	48.56	÷ 0.15	48.41
65	0.042	104.10	8.981	15.341	126.59	1.2160	62.02	51.00	÷ 0.65	50.35
39	0.028	101.97	4.342	14.578	124.03	1.2163	61.92	50.91	÷ 0.45	50.46
16	0.018	101.18	2.564	14.536	127.15	1.2567	64.27	51.14	+ 0.03	51.17
02	0.015	100.84	1.711	13.831	123.10	1.2207	61.45	50.34	+ 0.05	50.39
86	0.011	100.50	0.901	16.672	151.40	1.5065	75.32	50.00	+ 0.43	50.43
83	0.011	100.48	0.841	13.221	120.52	1.1994	61.84	51.56	+ 0.23	51.79
80	0.009	100.31	0.4328	16.306	149.21	1.4875	70.85	47.63	+ 1.15	48.78
76	0.009	100.31	0.4136	13.093	120.43	1.2006	56.22	46.83	+ 0.35	47.18

nonium sulphate concentration ($h = 5 \cdot 10 \div 6$).

alues of z, y and V_s corresponding with nd values of h and S.			The *inner* liquid then contains per 100 g. water			$\varepsilon = \frac{E}{V_s}$.	*Osmotic pressure* in cm. water pressure			
	y	V_s	Ammonium sulphate in g. S_i	Egg-hydrate in g. E_i	Milligr.-equival. protein nitrogen, $E = \frac{E_i \cdot 1000}{14.01 \cdot z}$		P (measured).	$\pi = \frac{P}{\varepsilon}$.	Correction in cm., to be added to π in order to refer the measurement to $h = 5 \cdot 10 \div 6$.	*Corrected* value of π (corresponding with $5 \cdot 10 \div 6$).
. 38 66).	(Fig. 37 p. 365).	(Tab. 32 p. 158).								
59	0.042	109.65	20.279	16.726	137.38	1.2529	58.50	46.69	÷ 1.20	45.49
58	0.043	108.81	18.562	17.383	142.94	1.3137	63.02	47.97	÷ 0.32	47.65
59	0.044	107.39	15.795	16.119	132.39	1.2328	62.77	50.92	÷ 0.17	50.75
59	0.045	106.65	14.330	17.042	139.98	1.3125	70.10	53.41	÷ 0.45	52.96
71	0.048	106.38	13.810	16.679	136.68	1.2848	67.51	52.55	+ 2.10	54.65
76	0.052	104.61	10.392	18.111	147.57	1.4107	78.51	55.65	÷ 0.90	54.75
74	0.055	101.99	4.363	15.009	122.57	1.2018	69.95	58.20	+ 0.45	58.65

the other two. Further, as may also be seen from the figure, we have at this hydrogen-ion concentration effected measurements at higher ammonium sulphate concentrations than those used at the other two. Indeed, in the two experiments concerned (No. 98 and No. 104, Table 55) the ammonium sulphate concentration was so high that the inner liquid, after being taken out

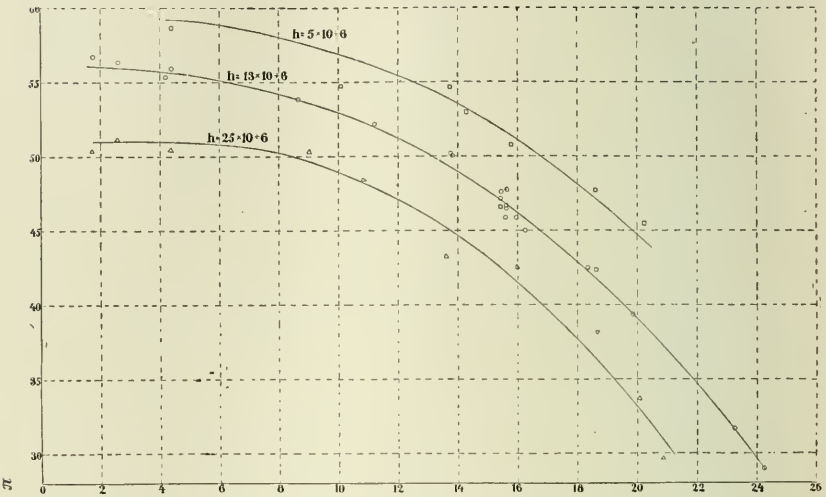

Fig. 33.

of the osmometer, separated in the course of the electrometric measurement a part of the egg-hydrate in crystalline form; during the osmotic measurement, however, the solution showed no crystals at all, and the continuous course of the curve bears evidence to the perfect accordance existing between the measurement results of these two experiments and those obtained in the other experiments of the same series.

As for the other two curves, and more particularly the uppermost, the experimental data are comparatively scanty, it is true, yet there can be no doubt that the three curves are practically parallel to one another, from which we may infer that under the conditions of our experiments the influence of the ammonium sulphate concentration on the osmotic pressure is independent of the hydrogen-ion concentration.

As regards the magnitude of the osmotic pressure in egg-albumin solutions containing minute portions of ammonium sulphate, we have hitherto made but a few experiments, the results

of which we are as yet unable to account for satisfactorily, and which we will therefore mention very succinctly. These experimental results are set forth at foot of each of the Tables 55 and 56. The corrected values of π, as found in the four experiments referred to, seem to indicate — more especially in Table 56 — that π declines a little with the ammonium sulphate concentration. On the other hand, a series of experiments described in Section *E* (p. 347) go to show that the osmotic pressure of an egg-albumin solution containing practically no ammonium-sulphate is far superior to that exerted by the ammonium-sulphate-containing solutions dealt with in this chapter. It will not be possible without further experiments to account for these apparently incompatible results, and we have therefore not entered the above eight experiments in Fig. 33.

The main result of the experiments dealt with in this section may, we believe, be formulated thus: *In the conditions of ammonium sulphate and hydrogen-ion concentration under which our experiments were made, π (the osmotic pressure per milligram-equivalent of protein-nitrogen per cubic-centimeter of dispersion medium) diminishes with increasing ammonium sulphate concentration. It is only with a fairly abundant content of ammonium sulphate (S = about 8) that this falling off begins to gather way; but thereafter it increases rapidly with increasing ammonium sulphate concentration.*

What can be the reason why the osmotic pressure decreases as the ammonium sulphate concentration increases? In the introduction (p. 293) we have pointed out how the measured osmotic pressure is influenced by the dissociation of protein salt in connection with the "Donnan counter-pressure". These two factors were shown to diminish that pressure, at least to a certain extent, as the ammonium sulphate concentration increased, under otherwise equal circumstances. At first sight, one might therefore be tempted to attribute the diminution of π to such a combined action of protein salt dissociation and "Donnan counter-pressure". But after a second reflection this explanation proves to be rather improbable. In fact, it was pointed out that — no matter whether the ammonium sulphate concentration be high or low — the aforesaid two factors never exert any influence at the proper hydrogen-ion concentration, which is probably not very different from that corresponding with the undermost curve of Fig. 33.

But the same cannot *a priori* be supposed to be the case with those experiments which were made at lower hydrogen-ion concentrations, and which are represented by the upmost two curves of Fig. 33, and yet these are nearly parallel to the undermost curve. The parallelism of the three curves seems to suggest that the same explanation is applicable to all the three series of experiments, and we therefore consider that the factors in question are also of little or no consequence in the experiments represented by the upmost two curves. This view agrees indeed with the fact pointed out on p. 291, *viz.* that protein salt dissociation and Donnan counter-pressure acquire real significance only, or at least chiefly, at comparatively low ammonium sulphate concentrations. Thus, in the last eight experiments set forth in Tables 55 and 56, the ammonium sulphate concentration is probably so insignificant that the factors referred to must necessarily be taken into account, so that this appears to be the way in which the before mentioned, seemingly contradictory results are to be accounted for (see also Section *E*, p. 353).

The principal cause of the diminution of osmotic pressure observed as the ammonium sulphate concentration increases under the conditions of the experiments described in the present section is in our opinion to be sought in a decreasing degree of dispersity of the disperse phase or, in other words, *in an increasing condensation of two or perhaps more egg-albumin particles to one single particle.* We imagine that *this condensation is brought about by means of the divalent SO_4 group, and, since the curves of Fig. 33 show the osmotic pressure of the solution to be reduced to about one half when the amount of ammonium sulphate increases in such a measure as to give rise to the crystallisation of the egg-hydrate (see p. 338), it is an obvious conclusion that the process may be simply supposed to consist in a condensation of two by two egg-hydrate particles, progressing with increasing ammonium sulphate concentration, and with formation of an egg-hydrate sulphate, which will thus crystallise out afterwards, when the concentration has attained a sufficient magnitude.*

If the hypothesis here set forth is adopted, it will be easily understood that at a given ammonium sulphate concentration the condensation must have advanced the farther the higher the hydrogen-ion concentration (within a certain limit, of course),

so that — as shown also by Fig. 33 — the osmotic pressure must be the lower the higher the hydrogen-ion concentration.

In support of the said hypothesis we may further mention that a number of measurements, which will be published in a following paper, have shown that the osmotic pressure of an ammonium-chloride-containing egg-albumin solution will diminish but very little if the proportion of ammonium chloride is augmented. We consider that this is due to the circumstance that in this case there is no divalent anion tending to knit together the egg-hydrate particles.

It also deserves notice that the view here taken of the crystallisation process agrees perfectly with those propounded in an earlier paper[1]) with regard to the composition of the egg-hydrate crystallised.

D. The influence of Hydrogen-ion concentration on the Osmotic Pressure.

In each of the two tables 58 and 59 we have placed together a series of experiments intended to elucidate the influence exerted by varying hydrogen-ion concentrations on the magnitude of the osmotic pressure of an egg-albumin solution under otherwise like conditions. The series shown in the former table, for which the ammonium sulphate concentration corresponding with $S = 15.6$ is chosen as the "normal", consists of sufficiently numerous experiments, we think, to justify us in considering the graph representing these (Fig. 34 I, the full-drawn curve) as a fairly adequate expression of the sought dependence at the ammonium sulphate concentration adopted as normal. As for the experiments set forth in Table 59, where the normal ammonium sulphate concentration corresponds with $S = 4.3$, they are, on the contrary, rather small in number, and are therefore represented by a dotted graph (Fig. 34 II).

The principles on which these two tables are elaborated are exactly the same as those underlying the preceding tables. To the values found for π (the third vertical column from the right) we have added a correction made out from the curves in Fig. 33, so that all the corrected values of π (last column) correspond with the normal ammonium sulphate concentration adopted for the

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 203 (1917).

TABLE 58.

Dependence of Osmotic Pre

No. of experiment.	Egg-albumin solution used.		Eksperimenter; No. of osmometer and No. of cap.	Found on analysis				Ammonium sulphate content of *outer*
	Mark.	Age, in days.		in 100 g. *outer* liquid	in 100 g. *inner* liquid		Hydrogen-ion concentration of *inner* liquid, h.	
				Ammonia nitrogen in g. (a_f).	Ammonia nitrogen in g. (a_b).	Protein nitrogen in g. (p_b).	$h \cdot 10^6$	
128	D. Æ. 4	216	Golds. III 3	2.8884	2.5492	1.4143	0.87	15.
165	»	690	Pal. IX 5	2.6501	2.3270	1.4927	4.54	14.
166	»	690	Pal. VII 7	3.3234	2.9084	1.4732	4.61	18.
127	»	216	Golds. II 6	2.8880	2.5519	1.4061	4.85	15.
106	»	87	Golds. III 3	2.5593	2.2587	1.4675	7.73	13.
97	»	58	Golds. II 6	3.2861	2.8746	1.4870	10.33	18.
90	I. D. Æ. 1	182	Golds. III 3	2.8659	2.4754	1.6429	10.45	15.
103	D. Æ. 4	80	Golds. II 6	2.9621	2.5983	1.4847	10.50	16.
88	I. D. Æ. 1	175	Golds. III 3	2.8559	2.4637	1.6534	10.96	15.
87	»	175	Golds. II 6	2.8636	2.4884	1.6046	11.14	15.
89	»	182	Golds. II 6	2.8632	2.4778	1.6315	11.51	15.
110	D. Æ. 4	129	Golds. III 3	2.5747	2.2663	1.4299	11.59	13.
112	»	174	Golds. III 3	3.3241	2.9358	1.3928	11.64	18.
111	»	174	Golds. II 6	2.5653	2.2577	1.4142	12.42	13.
164	I. D. Æ. 7	407	Pal. VII 7	2.8324	2.4468	1.6547	13.06	15.
137	»	18	Golds. II 6	2.8319	2.4648	1.5064	13.43	15.
61	I. D. Æ. 1	24	Chr. II 6	2.9169	2.5210	1.6319	13.58	15.
163	I. D. Æ. 7	407	Pal. IX 5	2.8382	2.4432	1.6775	14.45	15.
108	D. Æ. 4	96	Golds. III 3	3.3339	2.9207	1.4400	22.49	18.
116	»	185	Golds. III 3	2.9205	2.5654	1.3985	25.41	15.
123	»	209	Golds. II 6	2.8815	2.5445	1.4026	37.76	15.
124	»	209	Golds. III 3	2.8964	2.5448	1.4222	52.85	15.
125	»	214	Golds. II 6	2.8723	2.5286	1.4000	78.89	15.
126	»	214	Golds. III 3	2.8704	2.5323	1.4071	105.44	15.

Hydrogen-ion concentration ($S = 15.6$).

Values of z, y and V_s corresponding with and values of h and S.			The *inner* liquid then contains per 100 g. water				*Osmotic pressure* in cm. water pressure.			
z	y	V_s	Ammonium sulphate in g. S_i	Egg-hydrate in g. E_i	Milligr.-equival. protein nitrogen, $E = \frac{E_i \cdot 1000}{14.01 \cdot z}$	$\varepsilon = \frac{E}{V_s}$.	P (measured).	$\pi = \frac{P}{\varepsilon}$.	Correction, in cm., to be added to π in order to refer the measurement to $S = 15.6$.	*Corrected* value of π (corresponding to $S = 15.6$).
3.38 (66).	(Fig. 37 p. 365).	(Tab. 32 p. 158).								
71	0.047	107.39	15.790	16.268	133.31	1.2414	70.36	56.68	+ 0.20	56.88
69	0.045	106.65	14.330	17.042	139.98	1.3125	70.10	53.41	÷ 1.92	51.49
68	0.043	108.81	18.562	17.383	142.94	1.3137	63.02	47.97	+ 4.90	52.87
69	0.044	107.39	15.795	16.119	132.39	1.2328	62.77	50.92	+ 0.20	51.12
71	0.048	106.38	13.810	16.679	136.68	1.2848	67.51	52.55	÷ 2.55	50.00
69	0.043	108.69	18.337	17.561	144.24	1.3271	58.19	43.85	+ 4.52	48.37
69	0.044	107.32	15.654	19.262	158.21	1.4742	72.26	49.02	+ 0.02	49.04
69	0.044	107.63	16.272	17.224	141.47	1.3144	60.78	46.24	+ 0.95	47.19
69	0.044	107.29	15.586	19.394	159.29	1.4847	69.60	46.88	÷ 0.02	46.86
69	0.044	107.31	15.681	18.744	153.95	1.4346	68.00	47.40	+ 0.01	47.41
69	0.044	107.31	15.651	19.106	156.93	1.4624	69.28	47.37	+ 0.01	47.38
70	0.045	106.42	13.811	16.170	132.66	1.2466	63.19	50.69	÷ 2.50	48.19
69	0.043	108.81	18.602	16.331	134.14	1.2328	52.97	42.97	+ 4.90	47.87
70	0.046	106.40	13.724	15.956	130.91	1.2304	62.09	50.46	÷ 2.58	47.88
69	0.044	107.22	15.464	19.391	159.27	1.4885	69.99	47.12	÷ 0.30	46.82
69	0.044	107.21	15.342	17.376	142.72	1.3312	61.69	46.34	÷ 0.30	46.04
68	0.044	107.48	15.967	19.137	157.37	1.4642	66.81	45.63	+ 0.50	46.13
69	0.044	107.23	15.477	19.706	161.86	1.5095	70.95	47.00	÷ 0.22	46.78
67	0.042	108.85	18.584	16.917	139.27	1.2795	49.62	38.78	+ 5.05	43.83
66	0.042	107.49	15.874	15.963	131.57	1.2240	52.02	42.50	+ 0.58	43.08
65	0.039	107.37	15.735	15.980	131.86	1.2281	48.68	39.64	+ 0.18	39.82
62	0.035	107.42	15.770	16.175	133.93	1.2468	48.35	38.78	+ 0.31	39.09
5	0.028	107.34	15.611	15.720	131.23	1.2226	45.11	36.90	+ 0.08	36.98
9	0.022	107.33	15.644	15.689	131.91	1.2290	46.60	37.92	+ 0.08	38.00

TABLE 59.

Dependence of Osmotic Pres

No. of experiment.	Egg-albumin solution used.		Eksperimenter; No. of osmometer and No. of cap.	Found on analysis				Ammonium sulphate content of *outer*
	Mark.	Age, in days.		in 100 g. *outer* liquid	in 100 g. *inner* liquid		Hydrogen-ion concentration of *inner* liquid, h.	
				Ammonia nitrogen in g. (a_f).	Ammonia nitrogen in g. (a_b).	Protein nitrogen in g. (p_b).	$h \cdot 10^6$	S
133	D. Æ. 4	235	Golds. II 6	0.8889	0.7918	1.4386	5.50	4.3
95	»	52	Golds. II 6	0.8581	0.7590	1.5433	10.40	4.2
146	D. Æ. 7	72	Golds. III 3	0.8816	0.7779	1.4823	13.37	4.3
129	D. Æ. 4	224	Golds. II 6	0.8837	0.7829	1.4612	23.33	4.3
130	»	224	Golds. III 3	0.8803	0.7806	1.4613	36.31	4.3
131	»	230	Golds. II 6	0.8816	0.7728	1.4477	68.71	4.3
132	»	230	Golds. III 3	0.8817	0.7734	1.4438	106.9	4.3
134	»	235	Golds. III 3	0.8792	0.7751	1.4046	346.7	4.3

series concerned. We have not in these series either applied any correction for the difference of albumin concentration, since ε varies only from about 1.2 to 1.5. In Fig. 34 the corrected value of π is plotted as ordinate, the hydrogen ion-concentration h (eighth column) as abscissa.

It appears from the tables — and with especial clearness from Fig. 34 — that *the osmotic pressure of an egg-albumin solution does not undergo any material alteration if,* under otherwise equal conditions, *h varies between $40 \cdot 10^{\div 6}$ and $100 \cdot 10^{\div 6}$,* yet the curves indicate a minimum between $60 \cdot 10^{\div 6}$ and $80 \cdot 10^{\div 6}$. *At higher hydrogen-ion concentrations than $100 \cdot 10^{\div 6}$ the osmotic pressure seems to increase very slowly with the hydrogen-ion concentration, while at hydrogen-ion concentrations lower than $40 \cdot 10^{\div 6}$ the pressure increases rapidly as the hydrogen-ion concentration decreases.*

The experiments described in this section have primarily for their object to procure materials for the construction of one or more curves enabling us to effect the correction of π within a

Hydrogen-ion concentration ($S = 4.3$).

Values of z, y and V_s corresponding with found values of h and S.			The *inner* liquid then contains per 100 g. water				*Osmotic pressure* in cm. water pressure.			
z (Fig. 38 p. 366).	y (Fig. 37 p. 365).	V_s (Tab. 32 p. 158).	Ammonium sulphate in g. S_i	Egg-hydrate in g. E_i	Milligr.-equival. protein nitrogen, $E = \frac{E_i \cdot 1000}{14.01 \cdot z}$	$\varepsilon = \frac{E}{V_s}$.	P (measured).	$\pi = \frac{P}{\varepsilon}$.	Correction in cm, to be added to π in order to refer the measurement to $S = 4.3$.	*Corrected* value of π (corresponding to $S = 4.3$).
8.74	0.055	101.99	4.363	15.009	122.57	1.2018	69.95	58.20	+ 0.04	58.24
8.67	0.050	101.92	4.215	16.098	132.53	1.3003	73.71	56.69	÷ 0.02	56.67
8.62	0.046	101.97	4.306	15.279	126.51	1.2407	69.17	55.75	+ 0.03	55.78
8.39	0.028	101.97	4.342	14.578	124.03	1.2163	61.92	50.91	+ 0.03	50.94
8.15	0.013	101.97	4.338	14.106	123.54	1.2115	61.80	51.01	+ 0.02	51.03
7.85	0	101.97	4.265	13.368	121.55	1.1920	56.51	47.41	+ 0.03	47.44
7.78	0	101.97	4.262	13.194	121.05	1.1871	59.14	49.82	+ 0.03	49.85
7.75	0	101.96	4.256	12.736	117.30	1.1505	57.90	50.33	+ 0.02	50.35

limited domain of hydrogen-ion concentration. At the same time we have of course been endeavouring to form an idea as to the cause of the alteration undergone by the osmotic pressure under changes of hydrogen-ion concentration. We are, however, fully aware that this rather complicated question cannot possibly be cleared up without a far larger amount of experimental material than that which is available to us, and we must therefore limit ourselves to a few elucidatory remarks.

In the foregoing chapter we have pointed out that the dissociation of protein salts and the "Donnan counter-pressure" do not play any prominent part at the ammonium sulphate concentrations here considered and at hydrogen-ion concentrations not too far from the value $25 \cdot 10^{\div 6}$. Supposing this result to be correct, it is evident that in the curves of Fig. 34 the sections corresponding with hydrogen-ion concentrations only a little higher or lower than $25 \cdot 10^{\div 6}$ simply express the osmotic pressure of the egg-albumin particles, and the fact that this pressure is not the same in the experiments corresponding

with the two curves of Fig. 34 is doubtless — in accordance with the considerations set forth in the foregoing section — due to the circumstance that the condensation into egg-albumin sulphate, under otherwise equal conditions, has advanced farther when the ammonium sulphate concentration answers to $S = 15.6$ than when it corresponds with $S = 4.3$.

When the hydrogen-ion concentration is considerably lower than $25 \cdot 10^{\div 6}$, the combined effect of the dissociation of the

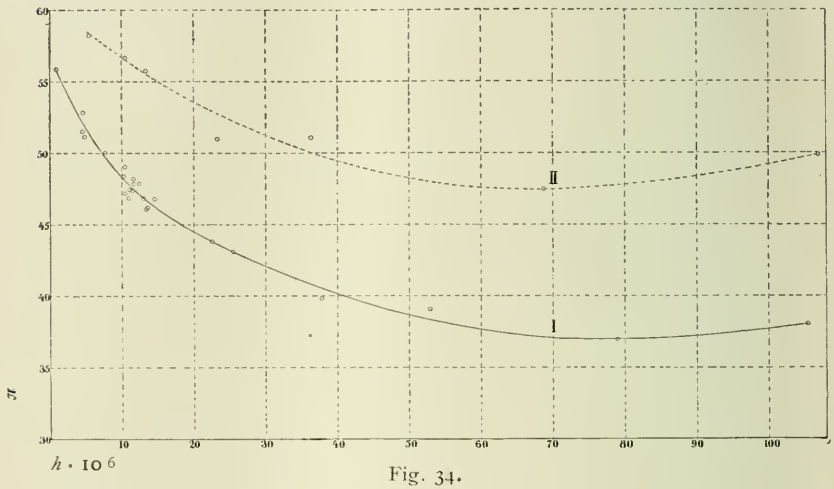

Fig. 34.

protein salt and of the "Donnan counter-pressure" may — as pointed out in the introduction to this paper (p. 292) — be of some moment, provided that the ammonium sulphate concentration is not too high; the effect alluded to will then make itself felt by an increase of osmotic pressure. This would account for the fact that the curves are ascending with decreasing hydrogen-ion concentration, if the solutions considered had very low contents of ammonium sulphate. But this is not the case, and, moreover, it appears by Fig. 34 that the curve corresponding with the highest ammonium sulphate concentration is the one which ascends most rapidly with diminishing hydrogen-ion concentration. We therefore consider that *the reason, or at any rate the main reason, of the ascent of the curves must be sought in a different direction, viz. in the circumstance that the condensation of the albumin-particles with formation of egg-albumin sulphate takes place in the less marked a degree as the hydrogen-*

ion concentration of the solution is lower (comp. p. 340). Indeed, this conjecture agrees perfectly with the fact that the ascent of the curve is most precipitous in the series of experiments where the ammonium sulphate concentration is highest, the extent of condensation being greater in this case.

The same theory will also account for the results arrived at when the hydrogen-ion concentrations are higher than $25 \cdot 10^{\div 6}$. The fact is, that *with increasing hydrogen-ion concentration the condensation proved to advance farther and, accordingly, the osmotic pressure to diminish: if, however, the hydrogen-ion concentration becomes much higher, the protein salt formation will also increase considerably, and the combined effect of protein salt dissociation and "Donnan counter-pressure" will then — in spite of the high ammonium sulphate concentration — give rise to an increase of osmotic pressure, which must be the greater as the ammonium sulphate concentration is lower.*

It would be highly interesting if, by measuring the osmotic pressure of egg-albumin solutions containing but a minimal amount of salt and having different hydrogen-ion concentrations, it could be shown that in such solutions where the protein salt dissociation and "Donnan counter-pressure" doubtless come into play, the state of things might be accounted for by the theory based upon Donnan's principles, as set forth in the introduction (p. 294); but we have not as yet set on foot any experiments in this direction.

E. The influence of Protein concentration on Osmotic Pressure.

If the osmotic pressure of an egg-albumin solution could be supposed, at a given composition of the dispersion medium, to be proportionate to the quantity of the disperse phase, then π — that is, the osmotic pressure expressed in centimeters of water pressure per milligram-equivalents of egg-albumin nitrogen per cubic centimeters of dispersion medium — would evidently be constant if the composition of the dispersion medium was kept constant. It was, however, not to be expected that things would prove to be so very simple as that, since the protein salt dissociation and the "Donnan counter-pressure" are generally — as mentioned in the introduction to this paper (p. 294) — de-

pendent on the concentration of the disperse phase. Neither can it be taken for granted *a priori* that the extent per cent of the albumin condensation is independent of the albumin concentration. We have therefore made this part of the matter also the object of experimental investigation; but the question is so exceedingly complicated that to clear it up entirely many more experiments would be required than those hitherto made.

The results arrived at in three series of experiments are put together in the usual manner in Table 60. The experimental arrangement was only in one point a little different from what it used to be. The fact is, that the composition of the dispersion medium had necessarily to be maintained constant, as far as possible, throughout all experiments belonging to the same series. We tried to attain this object with the help of an apparatus constructed on exactly the same principle as the osmometer, but of much larger dimensions. In this apparatus the egg-albumin solution, in as high a concentration as possible, was placed as inner liquid in a spacious collodion cap, while water or a suitable ammonium sulphate solution was used as outer liquid. The outer liquid was arranged to reach a somewhat higher level than the inner liquid in the cap. The latter was — like that of the osmometer — fastened on to a short glass tube, into which a ground glass-tube provided with a cock fitted very exactly. After the apparatus had been filled, the cock was closed. It was not our intention to undertake any measurement of the osmotic pressure; but when the cock had been closed, the outer liquid diffused gradually into the inner liquid, until the pressure produced by the expansion of volume established an equilibrium against the osmotic pressure of the inner liquid. After the apparatus had then been left to stand for some days in a well-regulated thermostat, while air saturated with toluol was passed continually through the outer liquid, we assumed the equilibrium to have been established and the outer liquid to have acquired practically the same composition as the dispersion medium of the inner liquid. In each experiment of this series we then used as outer liquids a part of the outer liquid obtained in the initial experiment, while using as inner liquids partly the inner liquid in the condition in which it was obtained, and partly after dilution with a greater or less portion of outer liquid. In this way we managed to keep the composition of the dispersion

medium practically constant within each series of experiments. so that the protein concentration remained the only variable factor.

The first series in Table 60, comprehending only four experiments, was carried out with a sample of non-dialysed egg-albumin solution. As seen in the eighth and ninth vertical columns of the table, the hydrogen-ion concentrations and the ammonium sulphate concentrations were nearly the same in each experiment, *viz.* h = about $13.7 \cdot 10^{\div 6}$ and S = about 15.4. The results set forth in the last column go to show that π, *under the conditions of these experiments, remains constant.* It is true that the result of Experiment No. 136 appears to differ considerably from the rest; but this discrepancy may probably be attributable to experimental errors, inasmuch as the albumin concentration in this experiment was so low that the osmotic pressure amounts to only about 20 cm. of water pressure.

The second series, consisting of six experiments, was made on a sample of dialysed egg-albumin solution to which was added some ammonium sulphate. The concentration of this substance was the same in all the experiments (S = about 4.36); but only in the last three experiments could the hydrogen-ion concentration be maintained constant. In the first three it was found to vary in a manner which was evidently due to some deficiency in their mode of arrangement. The variation also manifests itself in the value of π (last column). If, however, the values of π are corrected by the aid of the uppermost curve of Fig. 34 (p. 346), so that the measurements are referred to the hydrogen-ion concentration $13.5 \cdot 10^{\div 6}$ (mean value of h in the last three experiments), we arrive at the following values:

Experiment No. 149: $\pi = 47.54 \div 0.85 = 46.69$ cm
„ „ 150: $\pi = 53.93 \div 2.45 = 51.48$ „
„ „ 145: $\pi = 51.71 + 0.90 = 52.61$ „.

A comparison of these corrected values of π with the values found in the last three experiments will show that π under these conditions *increases continually aud very markedly as the protein concentration increases.*

The case is entirely the reverse in the last six experiments, where the ammonium sulphate concentration of the outer liquid is so low as to be practically nil. As regards the ammonia nitrogen contained in the inner liquid, it appears from the sixth column of

TABLE 60.

Dependence of Osmotic Press

No. of experiment.	Egg-albumin solution used.		Eksperimenter; No. of osmometer and No. of cap.	Found on analysis			
	Mark.	Age, in days.		in 100 g. *outer* liquid	in 100 g. *inner* liquid		Hydrogen-ion concentration of *inner* liquid.
				Ammonia nitrogen in g. (a_f).	Ammonia nitrogen in g. (a_b).	Protein nitrogen in g. (p_b).	$h \cdot 10$
							$S =$ about 1
136	I. D. Æ. 7	13	Golds. III 3	2.8342	2.7104	0.5033	14.0
135	»	13	Golds. II 6	2.8324	2.5936	1.0151	13.4
137	»	18	Golds. II 6	2.8319	2.4648	1.5064	13.4
138	»	18	Golds. III 3	2.8321	2.3651	1.9706	13.8
							$S =$ about 4
149	D. Æ. 7	84	Golds. II 6	0.8887	0.8662	0.3031	11.0
150	»	84	Golds. III 3	0.8841	0.8525	0.5031	6.4
145	»	72	Golds. II 6	0.8841	0.8346	0.7536	16.3
146	»	72	Golds. III 3	0.8816	0.7779	1.4823	13.3
148	»	78	Golds. III 3	0.8855	0.7344	2.1827	13.3
147	»	78	Golds. II 6	0.8863	0.6933	2.8415	13.7
							$S =$ abou
144	D. Æ. 7	37	Golds. III 3	less than 0.0001	0.0003	0.1465	10.4
142	»	32	Golds. III 3		0.0005	0.3055	12.5
140	»	27	Golds. III 3		0.0017	0.7388	16.4
139	»	27	Golds. II 6		0.0025	1.4671	17.42
141	»	32	Golds. II 6		0.0027	2.1657	19.45
143	»	37	Golds. II 6		0.0046	2.8051	18.79

Protein concentration.

Ammonium sulphate content of *outer* liquid in g. per 100 g. water. S	Values of z, y and V_s corresponding with found values of h and S. z (Fig. 38 p. 366).	y (Fig. 37 p. 365).	V_s (Tab. 32, p. 158).	The *inner* liquid then contains per 100 g. water: Ammonium sulphate in g. S_i	Egg-hydrate in g. E_i	Milligr.-equival. protein nitrogen, $E = \frac{E_i \cdot 1000}{14.01 \cdot z}$	$\varepsilon = \frac{E}{V_s}$	*Osmotic pressure* in cm. water pressure. P (measured).	$\pi = \frac{P}{\varepsilon}$
= about $13.7 \cdot 10 \div 6$									
15.429	8.69	0.044	107.22	15.400	5.278	43.35	0.4043	20.89	51.67
15.418	8.69	0.044	107.21	15.428	11.167	91.72	0.8555	40.20	46.99
15.415	8.69	0.044	107.21	15.342	17.376	142.72	1.3312	61.69	46.34
15.416	8.69	0.044	107.21	15.414	23.849	195.88	1.8271	85.15	46.60
= $(6.5-16.3) \cdot 10 \div 6$									
4.375	8.67	0.050	101.99	4.363	2.817	23.19	0.2274	10.81	47.54
4.351	8.75	0.055	101.97	4.359	4.805	39.20	0.3844	20.73	53 93
4.351	8.54	0.039	101.97	4.358	7.178	59.99	0.5883	30.42	51.71
4.338	8.62	0.046	101.97	4.306	15.279	126.51	1.2407	69.17	55.75
4.358	8.62	0.046	101.98	4.323	24.177	200.19	1.9631	119.12	60.68
4.362	8.61	0.046	101.98	4.336	33.793	280.14	2.7470	177.53	64.63
= $(10.4-19.5) \cdot 10 \div 6$									
0	6.45	0.007	100.136	0.0004	0.954	10.56	0.1055	12.26	116.21
»	»	»	»	0.0003	2.010	22.25	0.2222	22.43	100.95
»	»	»	»	0.0029	5.004	55.38	0.5530	53.64	97.00
»	»	»	»	0.0015	10.452	115.68	1.1552	99.29	85.95
»	»	»	»	÷0.0029	16.237	179.69	1.7945	138.93	77.42
»	»	»	»	0.0026	22.090	244.47	2.4414	204.81	83.89

the table to increase with the protein concentration, a result which — seeing that there is no ammonia nitrogen in the outer liquid — can hardly be accounted for in any other way than by supposing the ammonia of the inner liquid to be fixed by the albumin. If this assumption is correct, we are enabled, from the formula

$$y \cdot p_b = a_b \cdot 4.7163,$$

to find the factor, y, by which the weight of the protein nitrogen, p_b, of the inner liquid is to be multiplied to give the weight of the ammonium sulphate bound up with the albumin.

From the above formula we arrive at

$$y = \text{about } 0.008.$$

The reason why we have adopted the value 0.007 (eleventh column) lies in the circumstance that the dispersion medium of the inner liquid necessarily contains, like the outer liquid, a minute proportion of ammonia. Indeed, the thirteenth column shows that, if the value 0.007 is adopted as factor, a minute quantity of ammonium sulphate is left for the dispersion medium of the inner liquid, (S_i). An only exception is formed by Experiment No. 141, where this way of calculation gives a negative value of S_i, probably owing to an experimental error, which makes itself felt also in the value of π (last column) being different from that obtained in the other experiments. The hydrogen-ion concentration appears from the eighth column to increase with the protein concentration, which is quite natural inasmuch as the albumin is the only substance present in fairly abundant quantity; in this respect also Experiment No. 141 disagrees slightly with the others.

Apart from this experiment, it appears from the last column, first, that *the value of π decreases rapidly as the protein concentration increases*. and, secondly, that *π in this series attains far higher values than those found in all our previous experiments*. Seeing that the sample experimented upon was one of dialysed egg-albumin, it might be apprehended that this substance had in the course of dialysis undergone some alteration or other, say decomposition by bacterial action. But that no such thing has really happened is evident from the fact that, on being used *afterwards* — in the second series of Table 60; see first and third columns — with addition of a little ammonium sulphate, the same sample of dialysed egg-albumin showed a perfectly normal

osmotic pressure (Experiment No. 146 of the last-mentioned series accordingly fits perfectly in with the place assigned to it in Table 55, p. 334). Although this is the only series of experiments we have carried out on egg-albumin practically exempt from ammonium sulphate, yet we consider it justifiable to assert that *egg-albumin under such conditions shows a far higher osmotic pressure than it does in presence of fairly abundant quantities of ammonium sulphate.*

We have before stated our inability to furnish an exhaustive explanation of the interesting, though apparently contradictory results to which we have been led by the three experimental series just described; yet we think proper to subjoin a few remarks that may help the reader to take the bearings.

Let us first consider the undermost series, where the inner liquids were prepared from dialysed egg-albumin without admixture of ammonium sulphate, and may therefore by regarded as consisting mainly of a mixture of egg-albumin and an ammonium salt of the same[1]). The latter will in aqueous solution undergo a dissociation into ammonium ions and albumin ions, the degree of dissociation being the higher the lower the albumin concentration. Seeing now that — as shown by the simple calculation given below — any "Donnan counter-pressure" is out of the question, the dissociation supposed to have taken place will furnish a natural explanation, first, of the fact that the osmotic pressure in these experiments is higher than usual — since it is produced by the albumin particles as well as by the ammonium ions electrostatically fixed in the inner liquid —, and, secondly, of the fact that the osmotic pressure per unit of egg-albumin (π) increases with decreasing albumin concentration, since the degree of dissociation of the ammonium salt of the albumin increases with the dilution. Thus, we have here to do with phenomena similar to those met with in the limit case dealt

[1]) The dialysis of D. Æ. 7 lasted three weeks and was effected without addition of ammonia. As mentioned in a previous paper (Comptes-rendus du Laboratoire de Carlsberg **12**, 24 (1916), it is not possible in such a dialysis to remove every trace of the constituents of the ammonium sulphate, since egg-albumin, being an ampholyte of mainly acid character, is apt to bind some ammonia, but comparatively very little sulphuric acid. In our above considerations we disregard the sulphuric acid, the exact of amount of which we have not determined.

with in the introduction to the present paper, where c_2 is an infinitesimal quantity (see p. 295), and where the theory set forth demands the osmotic pressure measured to increase more slowly than the albumin concentration. Hence, qualitatively, our experimental results agree perfectly with the theory.

As regards the quantitative part of the matter, it is evident from the exceedingly low ammonia content of the outer liquids (less than one mg. ammonia nitrogen in one l) that a "Donnan counter-pressure" of measurable magnitude is out of the question, since the total amount of ammonia in the outer liquids corresponds with an osmotic pressure of less than $24685 \cdot \frac{1}{14.01 \cdot 1000} = 1.8$ cm. water-pressure (see p. 315). We have now to calculate the osmotic pressure exerted by the ammonia fixed per milligram-equivalent of egg-albumin nitrogen under the supposition of complete dissociation, and y' being put equal to 0.007. The ammonia nitrogen fixed per milligram-equivalent of egg-albumin nitrogen amounts to $\frac{0.007 \cdot 14.01}{4.7163}$ mg., and the osmotic pressure at 18^0 for an amount of ammonia nitrogen of 14.01 mg. per cubic centimeter is 24685 cm. water-pressure; consequently, the pressure sought will be

$$\frac{0.007 \cdot 14.01}{4.7163} \cdot \frac{24685}{14.01} = 36.6 \text{ cm. water-pressure.}$$

On the assumption of the ammonium salt being completely dissociated, the osmotic pressure of the egg-albumin particles is thus arrived at by subtracting 36.6 from the value found for π. In the case of Experiment Nr. 144 the calculation gives the result that the osmotic pressure of the egg-albumin particles amounts to as much as 116.21 ÷ 36.6 = ca. 79.6 cm. water pressure. But this value is probably too high; otherwise no material dissociation could be supposed to have taken place in Experiment No. 143, since the total pressure found here amounts to 83.89 cm. water-pressure. — Experiment No. 144 may also be subject to a considerable error, as the amount of albumin is here so minute that the osmotic pressure measured comes to only 12.26 cm. water-pressure. Yet, even if we disregard this experiment and suppose a complete dissociation to have taken place in Experiment No. 142, the osmotic pressure of the

albumin particles per milligram-equivalent protein-nitrogen will amount to $100.95 \div 36.6 = 64.35$ cm. water pressure, which is somewhat more than was found in our previous experiments.

We do not attach any decisive importance to these calculations, as they are only based upon a single series of experiments. But if the conclusions we have drawn should prove correct, at least approximately, the osmotic pressure of the egg-albumin particles per milligram-equivalent of protein-nitrogen thus being superior to the value arrived at in the preceding chapters, the result must evidently be accounted for by assuming either that the albumin condensation begins at a lower ammonium sulphate concentration than supposed by us, or that the "Donnan counter-pressure" exerts some influence even at such high ammonium sulphate concentrations as those in question.

If the latter assumption should turn out to be correct, the results obtained in the uppermost two experimental series of Table 60 can also be accounted for in a very simple manner. The osmotic pressure measured at not too low ammonium sulphate concentrations has been shown in the introduction (p. 295) to increase more rapidly than the albumin concentration, whilst at sufficiently high ammonium sulphate concentrations it increases in proportion to the albumin concentration. It is an obvious conclusion that the midmost experimental series of Table 60 ($S = 4.36$) corresponds with the former, the upmost series ($S = 15.4$) with the latter instance.

As mentioned before, the data obtained by the researches effected up to this time are insufficient to entirely clear up the rather complicated questions at issue; but if the explanation proposed is correct, there must evidently exist an intermediate ammonium sulphate concentration (corresponding with a value of S comprised between 0 and 4.36) at which the osmotic pressure measured increases in proportion to the albumin concentration, at which π will thus be constant and independent of the magnitude of ε. It will therefore be of considerable interest to carry out some additional investigations of the same kind as the three series represented in Table 60, but with different concentrations of ammonium sulphate.

In conclusion, we may mention that we have also considered the question as to whether the albumin concentration may exert any influence on the proportional extent of albumin condensation

in the case of a constant composition of the dispersion medium. As the condensation in question is one of two or more particles into one, it follows from the law of mass action as applied to this process, that the magnitude of the condensation will increase, and the osmotic pressure per milligram-equivalent of protein nitrogen consequently decrease with increasing albumin concentration. In this way the results set forth in the midmost series of Table 60 cannot therefore be accounted for.

On confronting the results exposed in Sections C, D and E it will be seen that we are as yet unable to give a definite answer to the question as to the value acquired by π under experimental circumstances where neither the albumin condensation nor the combined effect of albumin salt dissociation and "Donnan counter-pressure" come into play, so that the osmotic pressure may be reckoned as proportionate to the concentration of non-condensed egg-albumin. On the other hand, it is evident from those results that the value of π must under such circumstances be comprised between 60 and 70 cm. water-pressure. We are thus enabled to judge of the magnitude of the non-condensed albumin particles, inasmuch as π is equal to $\frac{RT}{n}$, where RT is equal to 24685 cm. water-pressure, and n means the number of nitrogen atoms in each albumin particle (see p. 315). As will easily be seen, *the calculation gives the result that, supposing π equal to 65 cm., n is about 380, so that the weight of an albumin particle — or molecule — in the anhydrous state will be approximately $380 \cdot 14.01 \cdot 6.45 =$ about 34000.*

If this result is confronted with the assumption (p. 340) that, as the ammonium sulphate concentration increases, there occurs a condensation of two by two egg-hydrate particles with formation of an egg-hydrate sulphate, which will crystallise out when formed in quantity sufficient for crystallisation, — and if it is further remembered that the sulphuric acid normally contained in the albumin crystallised amounts approximately to one equivalent of sulphuric acid to each 125 atoms of protein nitrogen[1]), then it will be admitted, we believe, that some countenance is thus lent to the conjecture that the *egg-albumin crystals normally consist of two*

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 203 (1917).

albumin particles bound up together by means of three molecules of sulphuric acid, this being the proportion of sulphuric acid which corresponds with about $125 \cdot 6 =$ about 750 atoms of protein nitrogen, while two albumin particles contain $380 \cdot 2 =$ about 760 atoms of protein nitrogen.

F. Determination of the quantities r, x, y and z.

With respect to the significance and calculation of these quantities we may refer to the explicit account given in a preceding paper[1]) of the theory and application of the Method of Proportionality.

Determination of r. As in all the experiments described in the present paper the quantity of albumin contained in the outer liquid is of no moment at all as compared with that in the inner liquid, it will be possible for each experiment to deduce the value of r from the reduced formula:

$$r = \frac{100\,(a_f \div a_b)}{a_f \cdot p_b},$$

with the aid of the values of a_f, a_b and p_b stated in Tables 54 to 60.

The values of r thus arrived at for all the experiments entered in Tables 55 to 59 are grouped together in Table 61.

The Sections A and B of this table evidence that, providing the ammonium sulphate concentrations are fairly high ($S >$ abt. 10), the magnitude of r is practically constant, but diminishes rapidly at decreasing concentrations. From Sections D and E it appears that at the high ammonium sulphate concentration ($S =$ about 15.6) the value of r is practically unaffected by the hydrogen-ion concentration, whereas at the low ammonium sulphate concentration ($S =$ about 4.3) we observe a marked increase with increasing hydrogen-ion concentration. Fig. 35 (p. 360) shows this dependence between r and h to be still more pronounced at still lower ammonium sulphate concentrations.

In this figure, where h is plotted as abscissa against r as ordinate, Curves I and II represent Sections D ($S =$ about 15.6) and E ($S =$ about 4.3) of Table 61, respectively, while Curves III (corresponding with $S =$ about 0.9) and IV (corresponding with $S =$ about 0.4) are based upon the last-mentioned

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 39 (1916).

TABLE 61.

Value of r at different Ammonium sulp

No. of experiment.	S	$h \cdot 10^6$	r
A. Different ammonium sulphate concentrations $h =$ abt. $13 \cdot 10^{\div 6}$ (corresponding with Table 55).			
98	24.262	10.33	8.49
104	23.266	10.21	8.39
62	19.841	12.22	8.55
112	18.592	11.64	8.39
97	18.341	10.33	8.42
103	16.239	10.50	8.27
61	15.951	13.58	8.32
90	15.629	10.45	8.29
87	15.614	11.14	8.17
89	15.612	11.51	8.25
88	15.566	10.96	8.31
163	15.455	14.45	8.30
164	15.418	13.06	8.23
137	15.415	13.43	8.61
110	13.822	11.59	8.38
111	13.764	12.42	8.48
105	11.192	10.86	8.10
96	8.638	10.52	7.89
146	4.338	13.37	7.94
95	4.217	10.40	7.48
94	2.570	11.27	6.85
93	1.717	10.12	6.20
82	0.895	14.45	5.79
92	0.849	11.32	5.74
81	0.444	15.67	4.48
91	0.437	10.84	3.59
B. Different ammonium sulphate concentration $h =$ abt. $25 \cdot 10^{\div 6}$ (corresponding with Table 56			
117	20.842	24.83	8.49
114	20.070	23.50	8.48
108	18.657	22.49	8.61
116	15.974	25.41	8.69
113	13.609	23.07	8.32
115	10.848	24.49	8.05
107	9.002	22.59	8.27
129	4.349	23.33	7.81
122	2.541	25.06	6.91
121	1.715	25.23	7.31
84	0.898	26.79	6.49
120	0.842	25.83	6.60
83	0.438	29.51	6.26
119	0.419	26.36	6.32

d Hydrogen-ion concentrations.

No. of experiment.	S	$h \cdot 10^6$	r
C. Different ammonium sulphate concentrations $h =$ abt. $5 \cdot 10^{\div 6}$ (corresponding with Table 57).			
167	20.228	3.78	8.31
166	18.588	4.61	8.48
127	15.768	4.85	8.28
165	14.284	4.54	8.17
106	13.727	7.73	8.00
68	10.076	4.06	6.67
33	4.376	5.50	7.59
D. Different hydrogen-ion concentrations $S =$ abt. 15.6 (corresponding with Table 58).			
28	15.771	0.87	8.30
65	14.284	4.54	8.17
66	18.588	4.61	8.48
27	15.768	4.85	8.28
06	13.727	7.73	8.00
07	18.341	10.33	8.42
00	15.629	10.45	8.29
03	16.239	10.50	8.27
38	15.566	10.96	8.31
07	15.614	11.14	8.17
09	15.612	11.51	8.25
10	13.822	11.59	8.38
112	18.592	11.64	8.39
111	13.764	12.42	8.48
164	15.418	13.06	8.23
137	15.415	13.43	8.61
61	15.951	13.58	8.32
163	15.455	14.45	8.30
108	18.657	22.49	8.61
116	15.974	25.41	8.69
123	15.727	37.76	8.34
124	15.822	52.85	8.54
125	15.669	78.89	8.55
126	15.657	105.44	8.37
E. Different hydrogen-ion concentrations $S =$ abt. 4.3 (corresponding with Table 59).			
133	4.376	5.50	7.59
95	4.217	10.40	7.48
146	4.338	13.37	7.94
129	4.349	23.33	7.81
130	4.332	36.31	7.75
131	4.338	68.71	8.52
132	4.339	106.9	8.51
134	4.326	346.7	8.43

four experiments in Table 61, Section *A*, and the corresponding four experiments in Section *B*, together with some similar experiments not given in Table 61.

A closer inspection of Fig. 35 shows that the degree of exactness with which r has been determined, though not particularly high, is tolerably good and at any rate sufficient to place it beyond doubt that the curves furnish a fairly adequate ex-

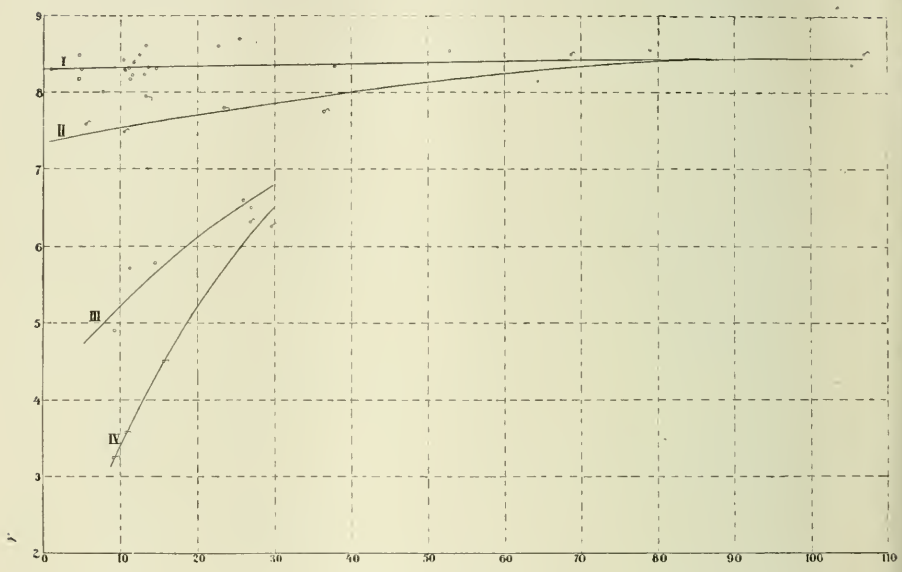

Fig. 35.

pression of the dependence existing between r and h at the ammonium sulphate concentrations concerned. Nor was a much greater exactness to be expected if it is taken into consideration that the magnitude of r depends chiefly on the *difference* between the ammonia amount of the outer liquid and that of the inner liquid. The shape of the curves can be easily accounted for if it is borne in mind that the decrease of r is due to the ammonia fixed by the albumin and will, accordingly, be the more considerable the larger the amount fixed and, more especially, the larger this amount is in proportion to the whole of the ammonia present. The amount fixed by the albumin diminishes with increasing hydrogen-ion concentration and becomes almost nil when the latter attains a sufficient magnitude, so that r gets a constant

value, which remains unaffected by the ammonium sulphate concentration; at high hydrogen-ion concentrations all the curves in Fig. 35 will therefore coincide, and, indeed, it is apparent from the figure that a production of the curve sections III and IV will coincide with the curves I and II. At low hydrogen-ion concentrations, on the contrary, the amount of ammonia fixed by the albumin cannot be disregarded, and the value of r will therefore decrease down to a level which will be the lower the less the total amount of ammonia.

In a similar way to that mentioned in Sections *B*, *C* and *D* in regard to the osmotic pressure, it is now possible to refer the values of r calculated direct from our experimental results to one and the same hydrogen-ion concentration, by adding to r the proper correction as derived from the curves in Fig. 35; any variation occurring in the values thus corrected must — experimental errors apart — be exclusively due to differences of ammonium sulphate concentration (see also below). As far as those experiments are concerned where the concentrations of hydrogen-ions and ammonium sulphate are sufficiently large, the corrections are of little or no account; but in the case of low concentrations they cannot be dispensed with.

We have thus corrected the values of r calculated immediately from our experimental results, so that the corrected values correspond with the hydrogen-ion concentrations $10 \cdot 10^{\div 6}$, $16 \cdot 10^{\div 6}$ or $25 \cdot 10^{\div 6}$ (in these series we have of course included only those experiments where the hydrogen-ion concentration is in the neighbourhood of one of these values). The numerical results are graphically reproduced in Fig. 36, where the corrected value of r is plotted as ordinate, S as abscissa, while Curve I (with points marked □) correspond with $h = 25 \cdot 10^{\div 6}$, Curve II (points marked ○) with $h = 16 \cdot 10^{\div 6}$, and Curve III (points marked △) with $h = 10 \cdot 10^{\div 6}$.

It appears by Fig. 36 — *conformably to the results just set forth* — that *the magnitude of r, at high concentrations of ammonium sulphate, is independent of the hydrogen-ion concentration and very nearly constant (about 8.4). With diminishing ammonium sulphate concentration the value of r begins to decrease, at first very slowly, but afterwards more and more rapidly, especially at lower hydrogen-ion concentrations.* In this connection it is to be borne in mind that the unequal distribution of the ammonium sulphate

between the dispersion medium of the outer and that of the inner liquid causes r to be found too high. The discrepancy due to that distribution is, however — as shown in the introduction to this paper (p. 291) — scarcely of any appreciable consequence at relatively high ammonium sulphate concentrations.

Finally, it is to be noticed that, since the quantity of ammonia fixed by the albumin is of the greater account the lower

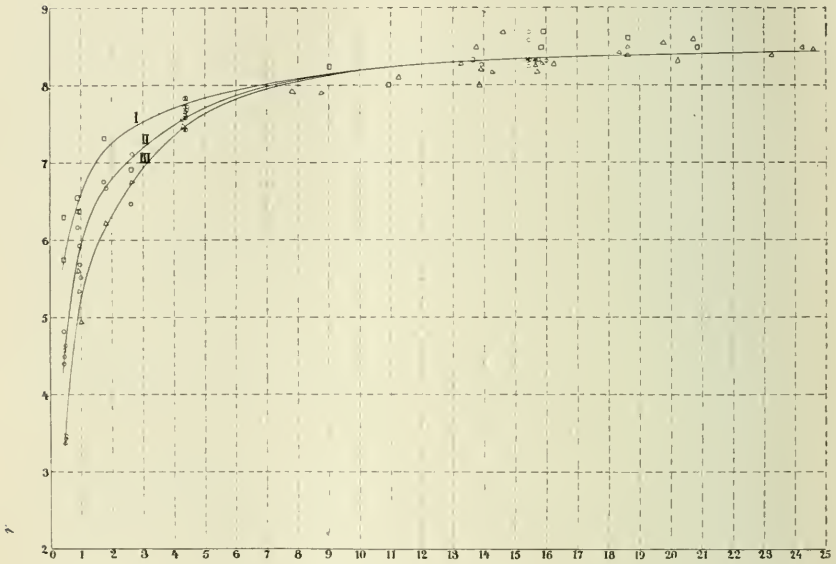

Fig. 36.

the ammonium sulphate concentration, it follows that at very low ammonium sulphate concentrations the difference $a_f \div a_b$ will approach to zero and at last become negative; in such cases the determination of r will be subject to error, and the values arrived at cannot be used for the below-mentioned calculation of y.

Determination of y, z and x. We have pointed out in a foregoing paper[1]) that it is possible to find y from the equation

$$y = \frac{r_1 \div r_2}{s_2 \div s_1},$$

where r_1 and r_2, corresponding with given values of S, can be

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 42 (1916).

found by the aid of Fig. 36[1]), while s_1 and s_2 can be calculated. In fact, we have

$$s = \frac{100\ (p_b \div p_f)}{4.7163\ (a_f \cdot p_b \div a_b \cdot p_f)},$$

and as p_f in all the cases here considered can be put equal to zero, we arrive at

$$s = \frac{100}{4.7163 \cdot a_f}$$

That is to say, s is equal to the ratio of the weight of the outer liquid to that of the ammonium sulphate contained in it; hence we may put

$$s = \frac{100 + S}{S},$$

since this fraction is but another expression of the said ratio.

If now, substituting two arbitrarily chosen values of S (S_1 and S_2), we calculate s_1 and s_2 and read off from Fig. 36 the corresponding values of r_1 and r_2, it is evident that y, as found from the formula

$$y = \frac{r_1 \div r_2}{s_2 \div s_1},$$

does not correspond with either of the two ammonium sulphate concentrations S_1 and S_2, but very nearly with the concentration $\frac{1}{2} \cdot (S_1 + S_2)$.

In Table 62 are placed together several values of y calculated in the way here exposed, the magnitude of r being determined by means of the midmost curve (corresponding with $h = 16 \cdot 10 \div 6$) of Fig. 36, so that the tabulated values of y correspond with this hydrogen-ion concentration.

The first three vertical columns are self-explaining, and the values of y calculated from them are stated in the fourth column. These values are plotted as ordinates, and the corresponding values of S as abscissæ, of Curve II, Fig. 37, from which the values of y as given in the fifth column of Table 62 were derived.

[1]) Seeing that the calculation of y hinges on the difference between r_1 and r_2, it would of course be a great mistake to use the direct found values of r, which are subject to considerable errors; it is necessary to have recourse to the values derived from Fig. 36, where the errors have been neutralised in the graphical representation.

TABLE 62.

S (g.$(NH_4)_2SO_4$ per 100 g. water).	$s = \frac{100 + S}{S}$.	r (read on Fig. 36, Curve II).	$y = \frac{r_1 \div r_2}{s_2 \div s_1}$.	y (read on Fig. 37, Curve II).	$s \cdot y$.	$z = r + s \cdot$
0.5	201.0	4.78		0.0118	2.37	7.15
			0.0102			
0.6	167.7	5.12		0.0125	2.10	7.22
			0.0151			
0.7	143.9	5.48		0.0132	1.90	7.38
			0.0128			
0.8	126.0	5.71		0.0140	1.76	7.47
			0.0137			
0.9	112.1	5.90		0.0147	1.65	7.55
			0.0108			
1.0	101.0	6.02		0.0154	1.56	7.58
			0.0150			
1.2	84.33	6.27		0.0169	1.43	7.70
			0.0168			
1.4	72.43	6.47		0.0183	1.33	7.80
			0.0146			
1.6	63.50	6.60		0.0197	1.25	7.85
			0.0173			
1.8	56.56	6.72		0.0212	1.20	7.92
			0.0198			
2.0	51.00	6.83		0.0227	1.16	7.99
			0.0180			
2.25	45.44	6.93		0.0245	1.11	8.04
			0.0225			
2.5	41.00	7.03		0.0263	1.08	8.11
			0.0247			
2.75	37.36	7.12		0.0281	1.05	8.17
			0.0297			
3.0	34.33	7.21		0.0299	1.03	8.24
			0.0336			
3.5	29.57	7.37		0.0335	0.99	8.36
			0.0392			
4.0	26.00	7.51		0.0372	0.97	8.48
			0.0432			
4.5	23.22	7.63		0.0408	0.95	8.58
			0.0450			
5.0	21.00	7.73		0.0436	0.92	8.65
			0.0440			
5.5	19.18	7.81		0.0455	0.87	8.68
			0.0464			
6.0	17.67	7.88		0.0466	0.82	8.70
			0.0462			
7.0	15.29	7.99		0.0472	0.72	8.71
			0.0472			
9.0	12.11	8.14		0.0464	0.56	8.70
			0.0450			
15.0	7.67	8.34		0.0436	0.33	8.67
			0.0412			
25.0	5.00	8.45				

If r, s and y are known, it is possible to calculate z, since

$$z = r + s \cdot y.$$

The values of z obtained are set forth in the last vertical column of Table 62, and used as ordinates against the corresponding

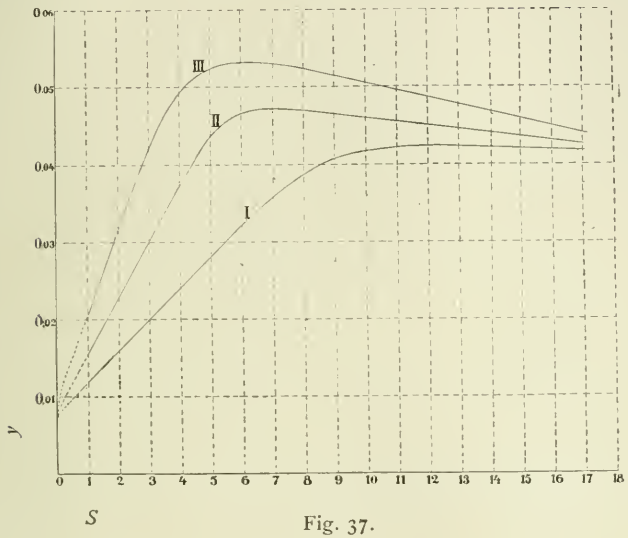

Fig. 37.

values of S as abscissæ of the Curve II of Fig. 38. — Finally, the factor x can be easily found, since

$$x = z \div y.$$

In the same manner as, with the hydrogen-ion concentration $16 \cdot 10 \div 6$, Curve II in Fig. 36 was used for constructing the curves relating to the same hydrogen-ion concentration in Fig. 37 and 38, we have in these figures by the aid of Curves I and III in Fig. 36 drawn up the corresponding curves for the hydrogen-ion concentrations $25 \cdot 10 \div 6$ (Curve I) and $10 \cdot 10 \div 6$ (Curve III), respectively.

Fig. 37 shows that, *with increasing ammonium sulphate concentration, the magnitude of y increases very considerably till it has reached its maximum value, whereafter it keeps constant.* As for the slight falling off exhibited in the value of y at high ammonium sulphate concentrations, it may possibly be due to experimental errors; but it is also possible that it has something to do with the condensation of the particles into egg-albumin

sulphate and the subsequent crystallisation. The fact is that — as pointed out in a foregoing paper[1]) — the crystallised egg-albumin may generally be supposed to contain some sulphuric acid, but very little ammonia, if any.

As regards solutions containing little or no ammonium sul-

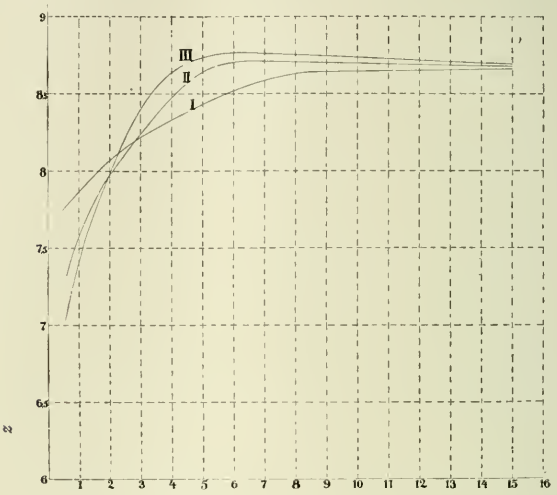

Fig. 38.

phate, we have seen above (p. 362) that y cannot be determined from the equation $y = \frac{r_1 \div r_2}{s_2 \div s_1}$, and in the foregoing section (p. 352) the amount of ammonia nitrogen contained in the outer liquid was shown in such cases to be almost nil, so that the ammonia of the inner liquid must be supposed to be almost entirely fixed by the egg-albumin. An approximately exact value of y will therefore in such cases be obtained from the following equation:

$$y = 4.7163 \frac{a_b}{p_b}.$$

It is, however, easy to find a formula enabling us to calculate y with greater exactness, if the dispersion medium of the inner liquid can be supposed to be of the same composition as the outer liquid. The fact is that 100 g. of inner liquid will contain (the designations being the same as before):

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**, 203 (1917).

$(p_b \cdot z)$ g. of egg-hydrate containing water and ammonium sulphate

$(a_b \cdot 4.7163 \div p_b \cdot y)$ g. ammonium sulphate, and

$(100 \div a_b \cdot 4.7163 \div p_b\ (z \div y))$ g. of water.

Thus, there is in the inner liquid, per 100 g. of water:

$$\frac{100\ (a_b \cdot 4.7163 \div p_b \cdot y)}{100 \div a_b \cdot 4.7163 \div p_b\ (z \div y)} \text{ g. of ammonium sulphate,}$$

which amount of ammonium sulphate we have above assumed to be equal to that existing per 100 g. of water in the outer liquid, that is equal to S. On this assumption, a simple calculation will give

$$y = 4.7163\ \frac{a_b}{p_b} \div \frac{S\ (100 \div p_b \cdot z)}{p_b\ (100 + S)}.$$

Accordingly, the above approximate value of y must be diminished by a correcting term: $\frac{S\ (100 \div p_b \cdot z)}{p_b\ (100 + S)}$, which, however, supposing S to be sufficiently low, is of no material account. The magnitude of z in this term is not known; but it is seen from Fig. 38 to decrease with decreasing S, and may therefore in the instance here considered be safely assumed to have its minimum value, 6.45.

By way of example of the calculation in question we may use the numerical results obtained in Experiment No. 143 (the last experiment in Table 60 (p. 350)). If $a_f = 0.0001$, we have $S = 0.0001 \cdot 4.7163 = 0.00047$; $a_b = 0.0046$; $p_b = 2.8051$; further, if z is put equal to 6.45, we arrive at

$$y = 4.7163\ \frac{0.0046}{2.8051} \div \frac{0.00047\ (100 \div 2.8051 \cdot 6.45)}{2.8051 \cdot 100.00047}$$

$y = 0.00773 \div 0.00014 = 0.00759$.

This example shows that the correcting term is of very little account, and that in the case considered it is quite immaterial whether the value of z is 6.45 or a little higher than this.

With the aid of the large apparatus described above (p. 348), which is not adapted for measuring the osmotic pressure, but by means of which an absolute equilibrium between inner and outer liquid can be established, we have, besides the experiments set forth in Table 60 (p. 350) with egg-albumin solutions containing very little ammonium sulphate, carried out some which allow a calculation of y by the method just exposed. With a

single exception (Experiment No. 141, Table 60; see also p. 352), the values of y were in all these experiments found to be comprised between 0.007 and 0.008, irrespective of the hydrogen-ion concentration of the solution, which varied from $10 \cdot 10^{\div 6}$ to $24 \cdot 10^{\div 6}$. Hence it would seem that this is no fortuitous result, and in Fig. 37 the three curves expressing the interdependence of y and S are therefore drawn in such a way as to converge towards the point $y = 0.007$ if $S = 0$. This is seen in the figure to tally perfectly with the general course of the curves. The section concerned is, however, represented by dotted lines because of the scantiness of our experimental material relating to ammonium sulphate concentrations inferior to those corresponding with $S = 1$.

As such egg-albumin solutions as those under consideration, which contain but a minimal amount of ammonium sulphate, are obtained by a prolonged dialysis *without* adjunction of ammonia, it follows that the two constituents of the ammonium sulphate are no longer present in equivalent proportions, but one of them, the ammonia, in comparatively much greater quantity than the other[1]). The former will be mainly present in the form of an ammonium salt of the albumin, and it will therefore be interesting to see what is the ratio of ammonia to albumin. If an egg-albumin particle — which has been shown (p. 356) to contain, probably, about 380 atoms of nitrogen — binds one molecule of ammonia, it is evident that the factor, y, by which the weight of the protein nitrogen has to be multiplied to give the weight of the amount of ammonium sulphate which corresponds with the ammonia fixed can be found from the equation

$$380 \cdot 14.01 \cdot y = 14.01 \cdot 4.7163,$$

which gives

$$y = \frac{4.7163}{380} = 0.0124.$$

Thus, since y has in the experiment just described been estimated at 0.007 to 0.008, it appears that the solutions examined do not even contain one molecule of ammonia to each albumin particle, but probably consist of a mixture of the albumin and its ammonium salt.

As for the magnitude of the factor z, it is shown by Fig. 38,

[1]) Comptes-rendus du Laboratoire de Carlsberg, **12**. 24 (1916).

at fairly high ammonium sulphate concentrations, to be nearly constant, practically equal to 8.7. If S is less than about 5, z will decrease with decreasing ammonium sulphate concentration, and the more so as the hydrogen-ion concentration is lower. The value or values to which z descends when S approaches to zero cannot be determined from the available data; but evidently it can never go down below 6.4[1]).

With regard to the variations which the factors r, y and z are found to undergo with the composition of the solution, we must admit that our graphical representations are subject to some uncertainty which cannot be left out of consideration; this is especially true of the curve sections relating to low ammonium sulphate concentrations. Still we confidently believe that the uncertainty is not, in general, sufficient to invalidate the application we have made of those curves.

It should also be borne in mind that the way in which we have attempted to clear up the state of things obtaining in colloidal solutions was never tried before, and we have therefore made a point of following up this course of reasoning to the farthest extent compatible with experimental results, even at the hazard of incurring the reproach that these are not sufficiently numerous or even exact enough in every particular to warrant each and every conclusion drawn from them.

SUMMARY.

Introduction.

1) The points of view from which the question as to the osmotic pressure of colloidal solutions has in our opinion to be considered are explained, and it is emphasised that the properties of colloidal solutions can be most efficiently inquired into by application, as far as possible, of the same views and methods as those generally applied to true solutions.

2) A brief account is given of some of the most important

[1] Comptes-rendus du Laboratoire de Carlsberg, **12**, 181 (1917).

researches hitherto published on the osmotic pressure of protein solutions.

3) The formulas proposed by Donnan concerning the state of equilibrium in the case of semi-permiable membranes are transformed in such a manner as to become applicable to protein solutions.

Section A.

The methods (including modes of calculation) and apparatus serving for determining the osmotic pressure of egg-albumin solutions are described in detail.

The osmometer used consists essentially of a collodion cap serving the purpose of a semi-permeable membrane and containing the egg-albumin solution employed as inner liquid, the outer liquid being represented by an ammonium sulphate solution in diffusion equilibrium against the dispersion medium of the inner liquid.

The osmotic pressure is measured on the compensation principle, that is, by determination of the counter-pressure required to be exerted on the surface of the inner liquid to prevent a migration of the liquids through the membrane.

The magnitude of the osmotic pressure, π, is expressed in centimeters water-pressure per milligram-equivalent of protein-nitrogen per cubic centimeters of dispersion medium.

The sources of error inherent to our method are exposed, more particularly the extent to which the exactness of the measurements is affected by the circumstance that the collodion cap, besides being slightly elastic, is not absolutely impermeable to egg-albumin.

Section B.

It is proved that an egg-albumin solution of given composition invariably exerts the same osmotic pressure.

Section C.

In this chapter are set forth several experiments intended to show how far the osmotic pressure of egg-albumin solutions is influenced by ammonium sulphate. According as the latter increases, π decreases, as far as the range of ammonium sulphate and hydrogen-ion concentrations experimented upon is

concerned. The decrease, however, is not very considerable unless the amount of ammonium sulphate is fairly large ($S =$ about 8), in which case the pressure diminishes rapidly as the ammonium sulphate concentration increases.

The principal cause of the diminution of osmotic pressure is sought in the circumstance that the increasing amount of ammonium sulphate tends to favour the condensation of two or perhaps more egg-hydrate particles into a single particle, by means of the divalent sulphate-group.

Section D.

This section is an account of some investigations into the influence exerted by hydrogen-ion concentration on the osmotic pressure of egg albumin solutions containing ammonium sulphate. The result arrived at is, that at hydrogen-ion concentrations comprised between $40 \cdot 10^{\div 6}$ and $100 \cdot 10^{\div 6}$ the osmotic pressure does not — under otherwise equal conditions — suffer any material alteration; at higher hydrogen-ion concentrations than $100 \cdot 10^{\div 6}$ the pressure appears to increase very slowly with the hydrogen-ion concentration, whilst at concentrations inferior to $40 \cdot 10^{\div 6}$ it increases rapidly when the concentration decreases.

These results, like those dealt with in the preceding section, are plausibly accounted for by the assumption of a condensation process promoted by the sulphate group.

Section E.

The influence of protein concentration on the magnitude of π is made the object of a few series of experiments, which go to show that π is only at ammonium sulphate concentrations, S, lower than 15.4 affected by the protein concentration. In a solution containing but a minimal amount of ammonium sulphate, π was found by a special series of experiments to decrease as the protein concentration increased, whereas another series, where S was equal to 4.36, showed π to increase with increasing protein concentration.

These results are also attempted to be traced back to the condensation process; yet in the case of solutions poor in ammonium sulphate the effect of the albumin salt dissociation and the Donnan counter-pressure are assumed to be concurrent

causes. The question, however, is rather too complex to be settled by the available experimental data.

The number of nitrogen atoms contained in a single non-condensed egg-albumin particle may, from the results exposed in the last three sections, be estimated with some probability at approximately 380; hence, the molecular weight of anhydrous egg-albumin appears to be about 34000.

Taking also into account the results arrived at in a foregoing paper with regard to the amount of sulphuric acid contained in the crystallised egg-albumin, the albumin crystals would seem to consist normally of 2 albumin particles bound up together by 3 molecules of sulphuric acid.

Section F.

The way in which the factors r, x, y and z are determined is explained, and their dependence on the ammonium sulphate and hydrogen-ion concentration exhibited by graphs.

November 1917.

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